IMPACTS DES ENSEMENCEMENTS ET DE L'INTROGRESSION GÉNÉTIQUE SUR LES POPULATIONS D'OMBLES DE FONTAINE (*SALVELINUS FONTINALIS*)

par

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SOMMAIRE

Les contacts entre animaux domestiques et sauvages sont fréquents dans le monde *via* les individus échappés des élevages, mais aussi *via* l'introduction volontaire d'individus d'élevage dans les populations naturelles pour des objectifs de conservation ou de gestion. Notamment, dans le domaine des pêcheries, les ensemencements de poissons de pisciculture sont courants, particulièrement chez les salmonidés, afin de soutenir des populations menacées ou de valoriser des plans d'eau pour la pêche récréative. La reproduction entre individus sauvages et ensemencés peut ainsi rapidement générer de l'introgression génétique (c'est-à-dire introduction d'allèles domestiques dans la population sauvage). Toutefois, le phénomène de domestication peut compromettre l'efficacité de ces mesures. En effet, la domestication peut modifier tant les caractéristiques génétiques que phénotypiques. Ses effets peuvent se manifester après seulement une génération de captivité et elle est inévitable dès lors que les individus sont maintenus dans un environnement artificiel. Lorsque les individus domestiqués sont confrontés à un environnement naturel, ils performent généralement moins bien que leurs conspécifiques sauvages sur de nombreux traits liés à l'aptitude phénotypique. L'objectif général de mon doctorat était donc de mieux caractériser les conséquences des ensemencements et de l'introgression génétique tant à l'échelle des individus que des populations. Pour ce faire, j'ai utilisé comme modèle d'étude l'omble de fontaine (*Salvelinus fontinalis*), un salmonidé très prisé pour la pêche récréative et massivement ensemencé depuis des décennies au Québec.

Dans un premier temps (Chapitre 2), je me suis intéressée aux effets potentiels de l'origine génétique des individus (c'est-à-dire domestiques, sauvages ou hybrides) sur leur phénotype. Pour cela, j'ai analysé d'une part la morphologie, et d'autre part la croissance et la taille à chaque âge des individus. J'ai également utilisé les isotopes stables pour caractériser la niche et le niveau trophique des individus selon leur origine génétique. Le phénotype, tant en termes de morphologie que de croissance ou de taille, semble être légèrement influencé par l'origine génétique, avec une tendance pour les domestiques à avoir une croissance plus importante. Cette

influence génétique semble toutefois s'exprimer différemment selon la population dans laquelle elle est étudiée, suggérant des interactions fortes entre les effets génétiques et environnementaux. De plus, l'identité du lac était le prédicteur le plus important du phénotype, indiquant que l'environnement dans lequel se trouvent les individus est le principal moteur de la variation de morphologie ou de croissance. En termes de niche et niveau trophique, les poissons domestiques se nourrissent dans des milieux plus benthiques et à des niveaux trophiques plus élevés que les deux groupes de poissons sauvages et hybrides qui ne présentaient entre eux que très peu de différences. Ces résultats suggèrent que les poissons domestiques pourraient s'approprier les niches trophiques les plus avantageuses lors de leur introduction. Ces résultats mettent donc en lumière le rôle de l'origine génétique sur le phénotype, mais aussi l'importance fondamentale de l'environnement à la fois sur le phénotype et sur l'expression des différences dues au bagage génétique.

Par la suite (Chapitre 3), je me suis intéressée aux impacts des ensemencements et de l'introgression sur les relations hôtes-parasites. Dans un premier temps, j'ai analysé les effets du bagage génétique au niveau individuel afin de déterminer si l'origine génétique pouvait influencer le statut infectieux ou l'intensité de l'infection. Aucune de ces deux variables n'était affectée par le bagage génétique des individus. Dans un second temps, je me suis intéressée aux effets populationnels des ensemencements en déterminant si le niveau d'introgression génétique était lié à la prévalence ou à la diversité de la faune parasitaire. Le niveau d'introgression avait un effet significatif négatif sur ces deux variables, indiquant que les lacs dans lesquels il y avait une plus grande quantité de gènes domestiques présentaient des prévalences plus faibles et un moins grand nombre d'espèces de parasites. Ces résultats indiquent donc un effet des gènes domestiques au niveau populationnel mais aucun impact au niveau individuel. Il semblerait donc que l'effet observé au niveau populationnel ne soit pas une conséquence directe de la présence des gènes domestiques. Ces résultats suggèrent donc que les lacs fortement ensemencés sont probablement des environnements de moins bonne qualité et les différences de prévalence et de diversité de faune parasitaire seraient attribuables à des effets environnementaux plutôt qu'à des effets dus aux ensemencements en tant que tels.

Enfin, je me suis intéressée aux effets des ensemencements et de leur intensité sur la taille effective (Chapitre 4). J'ai pour cela comparé les tailles effectives des lacs ensemencés une fois ou plus à celles des lacs n'ayant jamais été ensemencés. Les populations n'ayant jamais été ensemencées présentaient des tailles effectives significativement plus importantes que les populations ensemencées au moins une fois. Par la suite, j'ai utilisé les données des populations ensemencées au moins une fois afin de tester si l'intensité des ensemencements expliquait la taille effective. Pour quantifier l'intensité des ensemencements, j'ai utilisé quatre variables : 1) le nombre d'évènements d'ensemencements, 2) le nombre de poissons ensemencés par hectare, 3) le niveau d'introgression et 4) le nombre d'années depuis le dernier ensemencement. Ces quatre variables semblaient d'une manière générale avoir peu d'influence sur la taille effective, avec toutefois un léger effet négatif du niveau d'introgression. Par ailleurs, il y avait très peu de variabilité dans les valeurs de taille effective des lacs ensemencés. Les résultats indiquaient donc un effet important du statut d'ensemencement mais un effet plus faible de l'intensité des ensemencements. Ces résultats pourraient donc là encore s'expliquer par un effet de l'environnement avec les lacs ensemencés qui seraient moins productifs et auraient donc des tailles effectives plus faibles qui ne seraient pas une conséquence de l'introduction d'individus domestiques.

Les résultats de ma thèse suggèrent une importance modérée des ensemencements et de l'introgression génétique, tant au niveau populationnel qu'individuel. Ils permettent toutefois de souligner l'importance cruciale de l'environnement sur les variables étudiées. L'ensemble de ces informations permet de mieux comprendre les conséquences de l'introduction d'individus domestiques dans des populations sauvages. Les programmes d'ensemencements devraient donc tenir compte des impacts potentiels des introductions de poissons domestiques et adopter les stratégies de gestion de la ressource en conséquence.

Mots clés : ensemencements; introgression génétique; omble de fontaine; salmonidés; phénotype; parasites; taille effective.

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TABLE DES MATIÈRES

SOMMAIRE V

REMERCIEMENTS VIII

LISTE DES TABLEAUX XIII

LISTE DES FIGURES XVIII

CHAPITRE 1 INTRODUCTION GÉNÉRALE 1

Cadre théorique 1

Diversité génétique et tailles de populations 1

Ensemencements et domestication 4

Introgression génétique 10

Objectifs 15

Méthodes 16

Modèle d'étude et contexte des ensemencements dans le système 16

Données disponibles 17

CHAPITRE 2 PHÉNOTYPE 20

Description de l'article et contribution 20

Effects of genetic origin on phenotype in lakes stocked with domestic fish 21

Abstract 21

Introduction 22

Methods 26

Sampling and procedures 26

Genetic analyses 27

Morphometrics data 27

Growth data 28

Trophic niche and trophic level 29

Data and statistical analyses 29
Results 33
Morphometrics analyses 33
Growth analyses 34
Stable isotopes analyses 39
Discussion 43
Morphology 43
Growth 45
Trophic niche and trophic level 46
Conclusion 48
Acknowledgments 49
Funding 49
References 49
CHAPITRE 3 RELATIONS HÔTES-PARASITES 64
Description de l'article et contribution 64
Introgressive hybridization between wild and domestic individuals and its relationship with parasitism in brook charr *Salvelinus fontinalis* 65
Abstract 65
Introduction 66
Material and methods 69
Sampling sites and procedures 69
Parasitism analyses 70
Genetic analyses 74
Model variables and statistical analyses 75
Results 77
Individual analyses 78
Population analyses 80
Discussion 81
Individual level 81

Population level 83
Acknowledgements 85
References 86
CHAPITRE 4 TAILLE EFFECTIVE 98
Description de l'article et contribution 98
Impacts of stocking and its intensity on effective population size in Brook Charr (*Salvelinus fontinalis*) populations 99
Abstract 99
Introduction 100
Methods 103
Sampling sites and procedures 103
Genetic analyses 104
Ne estimates 109
Effects of stocking and its intensity 110
Results 112
Discussion 118
Effect of stocking on Ne 118
Effect of stocking intensity on Ne 120
Effect of stocking interruption on Ne 122
Estimating Ne in natural contexts 123
Conclusions and perspectives 125
Acknowledgments 126
Funding 126
Compliance with Ethical Standards 126
Conflict of Interest 127
Data availability 127
References 127
CHAPITRE 5 DISCUSSION ET CONCLUSION GÉNÉRALE 138
Discussion 138

Retour sur les résultats 138
Importance de l'environnement sur les variables étudiées 140
Pistes de recherche futures 142
Conclusion générale 146
ANNEXES 147
Annexes Chapitre 2 147
Appendix A: Supplementary information on methods and results. 147
Appendix B: Additional analyses results. 160
Annexes Chapitre 3 166
Appendix A: Supplementary analyses and information on methods and results. 166
Appendix B: References 173
Annexes Chapitre 4 175
Appendix A: Supplementary analyses and information on methods and results. 175
Appendix B: References 190
BIBLIOGRAPHIE 192

LISTE DES TABLEAUX

Table 1.1 **Différents types d'ensemencements en fonction du type d'élevage. Adapté de Araki et al. 2008. .....** 11

Table 1.2 **Définition des termes associés à l'origine et au bagage génétique des individus considérés dans cette étude. .....** 14

Table 2.1 **F-values from backward stepwise selection of linear models on morphometric data (relative warp analysis, n = 457). .....** 35

Table 2.2 **F-values from backward stepwise selection of linear models on growth (cm/year) and total length (cm) at each age ("YO" = years old). .....** 36

Table 2.3 **Results of the Tukey post-hoc tests of the effects of genetic origin on δ13C and δ15N ratios. .....** 40

Table 3.1 **Summary of information for sampled lakes in this study. .....** 72

Table 3.2 **Results of generalized linear mixed models at the individual (infection status) and population (prevalence) level and of generalized linear model at the population level (number of parasite species) in populations of Salvelinus fontinalis .....** 79

Table 4.1 **Characteristics of all hatcheries and sampled populations including their stocking history, genetic features and effective population sizes (NeLD, estimated with the linkage disequilibrium method). .....** 106

Table 4.2 **Final model (GLM, negative binomial distribution) assessing whether stocking status impacts effective population size (NeLD estimated with the linkage disequilibrium method, PCrit=0.05). .....** 113

Table 4.3 **Final models (GLM, negative binomial distribution) assessing whether stocking intensity is related to effective population size (NeLD estimated with the linkage disequilibrium method, PCrit=0.05).** 115

Table S2.A1 **List of lakes used for phenotypic analyses with respective effectives for analyses using morphometrics and growth data.** 148

Table S2.A2 **List of lakes used for stable isotopes analyses with their effectives and values of introgression.** 149

Table S2.A3 **Results of the Procrustes ANOVA using all lakes.** 150

Table S2.A4 **Results of the Tukey post-hoc tests of the effects of genetic origin on δ13C and δ15N ratios.** 151

Table S2.A5 **Results of ANOVA on δ13C data to determine if (a) genetic status and length (defined as length in cm between landmarks 2 and 11, see Fig. S2.A1) influence trophic niche for all fish, and if (b) length influence trophic niche in domestic fish only.** 152

Table S2.B1 **Results of the Procrustes ANOVA including only lakes from Saint-Maurice reserve.** 160

Table S2.B2 **F-values from backward stepwise selection of linear models on morphometric data (relative warp analysis, n = 330). Only lakes from Saint-Maurice reserve are in this analysis.** 161

Table S2.B3 **Results of the Procrustes ANOVA including only lakes from Saint-Maurice reserve and sex (males, females or indeterminate) is included as an explanatory variable.** 162

Table S2.B4 **F-values from backward stepwise selection of linear models on morphometric data (relative warp analysis, n = 330). Only lakes from**

Saint-Maurice reserve are in this analysis and sex (males, females or indeterminate) is included as an explanatory variable.....163

Table S2.B5 F-values from backward stepwise selection of linear models on growth and size at each age ("YO" = years old). Only lakes from Saint-Maurice reserve are in this analysis. 164

Table S2.B6 F-values from backward stepwise selection of linear models on growth and size at each age ("YO" = years old). Significant variables ($p < 0.05$) are in bold. Only lakes from Saint-Maurice reserve are in this analysis and sex (males, females or indeterminate) is included as an explanatory variable.....165

Table S3.A1 Final models after selection of generalized linear mixed models when considering external and internal parasites for individual analyses of Salvelinus fontinalis. 166

Table S3.A2 Final models after selection of generalized linear mixed models for prevalence analyses of external and internal parasites in populations of Salvelinus fontinalis. 167

Table S3.A3 Characteristics of microsatellite loci used to genotype Salvelinus fontinalis with their names, repeat motifs, allele size range, $MgCl_2$ concentration (mM) used in PCRs, annealing temperature and number of cycles used for PCR amplification and the references for further details about loci.....168

Table S3.A4 Prevalence (% of infected individuals) found for each parasite species and for external, internal and total parasites of Salvelinus fontinalis in the sampled lakes.....170

Table S3.A5 Values of expected (HE) and observed (HO) heterozygozity for Salvelinus fontinalis in each lake and domestic strains. 172

Table S4.A1 Characteristics of microsatellite loci used to genotype brook charr (Salvelinus fontinalis). 178

Table S4.A2 Correlations between the 3 methods of Ne estimation with the temporal method. 179

Table S4.A3 Pearson correlations between the parameters of intensity of stocking..... 179

Table S4.A4 Summary of effective population size (Ne) estimates obtained with all methods and their respective thresholds of rare alleles exclusion (PCrit). 180

Table S4.A5 Final model (GLM, negative binomial distribution) obtained after backward selection to assess the relationship between stocking status (stocked VS unstocked lakes) impacts effective population size (NeLD, estimated with the linkage disequilibrium method with PCrit=0.02)..... 184

Table S4.A6 Final models (GLM, negative binomial distribution) assessing whether stocking intensity is related to effective population size (NeLD estimated with the linkage disequilibrium method, PCrit=0.02). 185

Table S4.A7 Final model (GLM, negative binomial distribution) obtained after backward selection to assess the relationship between stocking status (stocked VS unstocked lakes) impacts effective population size (NeLD, estimated with the linkage disequilibrium method with PCrit=0.05) after the removal of populations having a strong linkage disequilibrium..... 186

Table S4.A8 Final model (GLM, negative binomial distribution) obtained after backward selection to assess the relationship between stocking status (stocked VS unstocked lakes) impacts effective population size (NeLD,

estimated with the linkage disequilibrium method with PCrit=0.02) after the removal of populations having a strong linkage disequilibrium. 187

Table S4.A9 Final models (GLM, negative binomial distribution) assessing whether stocking intensity is related to effective population size (NeLD estimated with the linkage disequilibrium method, PCrit=0.05) after the removal of populations having a strong linkage disequilibrium. 188

Table S4.A10 Final models (GLM, negative binomial distribution) assessing whether stocking intensity is related to effective population size (NeLD estimated with the linkage disequilibrium method, PCrit=0.02) after the removal of populations having a strong linkage disequilibrium. 189

Table S4.A11 Pearson correlation between lake area and a proxy of Brook Charr density for a subsample of 28 lakes of the study. Density was estimated as the number of fish caught in per net per hour during sampling (catch per unit effort, for details, see Gossieaux et al. 2018). 189

LISTE DES FIGURES

Figure 1.1 **Carte du site d'étude.** 18

Figure 2.1 **Extreme shapes of each PC from PC1 to PC4.** 37

Figure 2.2 **Graphs of significant interactions between q-value and lake at (A) 1 year old and (B) 4 years old, and cohort (i.e. hatching year) at (C) 4 years old and (D) 5 years old on total length.** 38

Figure 2.3 **Stable isotopes ratios of δ13C and δ15N for Brook Trout in four lakes.** ... 41

Figure 2.4 **Frequency of individuals of each genetic status along the δ13C gradient of each lake.** 42

Figure 3.1 **Location of sampled lakes in the wildlife reserves of Portneuf, Mastigouche and Saint-Maurice in Québec, Canada.** 70

Figure 3.2 **Relationships between the mean q-value (standardized, mean = 0, $\sigma^2 = 1$) of lakes and (a) parasite prevalence and (b) number of parasite species in Salvelinus fontinalis.** 80

Figure 4.1 **Generalized linear models predictions of how effective population size (NeLD) varies with proxies of intensity of stocking including (a) number of stocking events, (b) number of fish stocked per hectare, (c) proportion of domestic genes in a lake and (d) number of years since last stocking event.** 117

Figure S2.A1 **Placement of the landmarks for morphometric analysis.** 153

Figure S2.A2 **Sagittal cut of an otolith.** 154

Figure S2.A3 Decision tree of otolith reading. 155

Figure S2.A4 Illustration of the extremes forms of PC1. 156

Figure S2.A5 Mean back-calculated length (cm) at each age with 95% confidence intervals. 157

Figure S2.A6 Graphs of significant interactions between q-value and lake between (A) 0 and 1 year old and (B) 3 and 4 years old on growth (cm/year). 158

Figure S2.A7 Mean length (cm) with 95% confidence intervals for each genetic group along the δ13C gradient for each lake. 159

Figure S4.A1 Distribution of body length (length from the snout to the base of the caudal fin, cm) of sampled fish in each lake sampled in 2007-2008. 176

Figure S4.A2 Distribution of total length (cm) of sampled fish in cm in each lake sampled in 2014-2016. 177

CHAPITRE 1
INTRODUCTION GÉNÉRALE

Cadre théorique

Diversité génétique et tailles de populations

Un des enjeux majeurs actuels dans le domaine de l'écologie et de la conservation est le déclin de nombreuses populations animales et végétales à travers le monde. Déjà aujourd'hui, les taux d'extinction sont de 100 à 1000 fois plus élevés qu'avant l'apparition des civilisations humaines et les taux d'extinction futurs restent impossibles à prévoir avec précision (Pimm *et al.*, 1995). Les risques sont multifactoriels et compliqués à estimer étant donné que de nombreuses variables peuvent entrer en jeu dans l'extinction des populations et, ultimement, des espèces (Keith *et al.*, 2008). La taille des populations peut être réduite par un grand nombre de facteurs tels que la perte d'habitat, la surexploitation, les espèces envahissantes ou encore la pollution (Goossens *et al.*, 2006; Rosser et Mainka, 2002; Wilcove *et al.*, 1998). Suite à ces diminutions, il est possible que les effectifs des populations atteignent un point critique à partir duquel les facteurs stochastiques augmentent beaucoup le risque d'extinction (Shaffer, 1981; Spielman *et al.*, 2004).

Dans ces circonstances de déclin de nombreuses populations à une échelle globale, des politiques de conservation et de restauration émergent afin de limiter les impacts anthropiques sur les populations naturelles. Dans ce contexte, un des facteurs les plus importants à prendre en compte est la diversité génétique des populations (Reed et Frankham, 2003). Les conséquences des activités humaines comme la dégradation de l'habitat, la surexploitation ou

la translocation de populations peuvent avoir sévèrement affecté la composition et la structure génétique des populations naturelles au cours du temps (Bradshaw, 2004; Hansen *et al.*, 2009; Liu *et al.*, 2009; Mathisen, 1996). La diversité génétique est pourtant un paramètre capital de la persistance des populations, ainsi qu'une composante de la biodiversité (Reed et Frankham, 2003).

Des populations vivant dans des lieux distincts ne font pas forcément face aux mêmes pressions de sélection car leurs environnements respectifs peuvent être différents, ce qui peut générer des adaptations locales. Kawecki et Ebert (2004) ont établi que des organismes adaptés localement sont censés avoir une meilleure aptitude phénotypique dans leur environnement local que des migrants qui viendraient d'autres populations. Ainsi, des populations adaptées localement présenteraient entre elles des différences génétiques dues en partie à ces adaptations (Fraser *et al.*, 2011; Kawecki et Ebert, 2004; Taylor, 1991). Les adaptations locales sont par ailleurs importantes pour l'aptitude phénotypique et, même en cas de consanguinité, l'hybridation avec des populations exogènes peut être contre-sélectionnée afin de conserver ces adaptations, car les perdre serait plus délétère que de supporter un certain niveau de consanguinité (Verhoeven *et al.*, 2011). Une perte de diversité génétique inter-populationnelle peut entraîner une homogénéisation génétique et la perte des adaptations locales pourrait alors mettre en péril les populations à large échelle (Lacy, 1997; Olden *et al.*, 2004). Des effets inattendus pourraient aussi apparaître comme cela a été le cas chez les fourmis d'Argentine (*Linepithema humile*) qui, une fois introduites en Californie, ont perdu une grande part de leur diversité génétique suite à un effet fondateur. Cela a entraîné une modification importante de leur structure sociale, décuplant ainsi leur potentiel envahissant par rapport à d'autres populations de la même espèce (Tsutsui et Case, 2001). Les adaptations locales sont modulées par différents facteurs. L'un des plus importants est le flux de gènes qui peut ralentir ou empêcher l'apparition d'adaptations locales (Garant *et al.*, 2007; Kawecki et Ebert, 2004), bien que certaines populations puissent conserver leurs caractéristiques locales malgré un flux de gènes important pour peu que la sélection soit forte (Fitzpatrick *et al.*, 2015).

La diversité génétique d'une manière générale peut être modulée par de nombreux facteurs. Elle peut notamment être réduite par la consanguinité, les effets fondateurs, les goulots d'étranglement ou encore la dérive génétique. Ces phénomènes sont directement liés à la taille des populations. Ainsi, les effets fondateurs ou les goulots d'étranglement génèrent des populations de petite taille et plus une population est petite, plus elle est soumise à la dérive et à la consanguinité (Frankham *et al.*, 2004). Maintenir les populations à des tailles suffisantes pour éviter ces effets est donc souvent une priorité des programmes de conservation ou de gestion.

Les tailles de populations peuvent être évaluées selon différents critères. On peut globalement différencier deux catégories principales, d'une part les tailles obtenues par dénombrement des individus (ex. taille recensée, nombre de reproducteurs) et d'autre part la taille génétique des populations (Hamilton, 2009). Cette dernière est également appelée taille effective. Elle tient compte de la façon dont se comporte la variation génétique (Frankham *et al.*, 2004). Elle prend notamment en considération le nombre d'individus capables de se reproduire, le sex-ratio, la variation de succès reproducteur entre les individus et l'histoire évolutive de la population (ex. s'il y a eu des évènements qui ont pu drastiquement diminuer la variation génétique comme un goulot d'étranglement ou un effet fondateur) (Frankham *et al.*, 2004). La taille effective dépend donc de la façon dont la variation génétique change dans le temps. Elle est un paramètre clé en écologie car c'est une des mesures les plus pertinentes pour évaluer la capacité de réponse d'une population aux forces évolutives et pour établir l'importance de la dérive génétique (Waples, 2010), et elle est donc intimement liée à la diversité génétique. Plus la taille effective est grande, plus la probabilité de conserver la diversité génétique est élevée. Frankham *et al.* (1999) ont par exemple illustré le lien entre taille de population et diversité génétique en montrant qu'un goulot d'étranglement où on établit une nouvelle population avec un seul couple de mouches *Drosophila melanogaster* en une génération réduit l'hétérozygotie de 25% et donc diminue le potentiel évolutif de la population.

Ensemencements et domestication

La taille des populations étant un enjeu majeur en conservation et en gestion des populations exploitées, de nombreux programmes sont élaborés pour rétablir ou préserver des tailles de populations suffisantes (Laikre *et al.*, 2010; Soorae, 2013). Une méthode largement répandue est l'introduction d'individus dont la reproduction a été assurée et contrôlée en élevage. Ce procédé se pratique chez tous les taxa ou presque (Laikre *et al.*, 2010; Soorae, 2013). L'un des objectifs principaux de ces programmes d'introduction est d'augmenter les effectifs d'une population, notamment afin d'éviter des phénomènes de dérive pour conserver une bonne diversité génétique. Ces programmes ne sont pourtant pas anodins pour les populations ciblées et certains facteurs peuvent en altérer l'efficacité. L'un d'eux est le phénomène de domestication.

Price (1999) considère qu'il est difficile de donner une définition claire de la domestication, qui soit assez générale pour prendre en compte les processus multiples qui entrent en jeu tout en étant assez spécifique pour avoir un sens en termes évolutifs et biologiques. Elle peut toutefois être définie comme « un processus par lequel une population animale devient adaptée à l'homme et à l'environnement captif suite à des changements génétiques qui se produisent au fil des générations et à des événements développementaux induits par l'environnement et qui se reproduisent à chaque génération » (Price, 1999). Elle implique donc une population née et élevée en captivité qui est génétiquement distincte de la population ancestrale (Diamond, 2002). Les changements génétiques induits par la domestication sont plus quantitatifs que qualitatifs (c'est-à-dire il n'y a pas forcément apparition ou disparition de traits, mais plutôt des changements dans l'intensité de leur expression). Les traits avec une forte héritabilité peuvent donc être modifiés en assez peu de temps, qu'ils soient sélectionnés délibérément ou non (Price, 1999). En effet, les changements dus à la domestication peuvent être volontaires lorsqu'un trait d'intérêt est sélectionné, mais ils peuvent également être non voulus (Perry *et al.*, 2005). La domestication modifie donc le patrimoine génétique de la population captive et, à terme, les

individus domestiqués présentent des différences parfois importantes par rapport à leurs ancêtres sauvages. La domestication peut modifier la forme des organismes et, à partir d'une seule espèce ancestrale, de nombreux phénotypes différents peuvent apparaître en fonction de ce pour quoi ils ont été sélectionnés. C'est le cas notamment des chiens (*Canis lupus familiaris*) chez lesquels on peut observer des phénotypes extrêmement variés alors qu'ils sont tous issus d'un même ancêtre avant leur domestication (Diamond, 2002). La sélection liée à la domestication se faisant par l'action humaine et non par sélection naturelle, ces changements peuvent être contre-évolutifs s'ils sont évalués dans le contexte de la vie en milieu naturel.

La domestication peut affecter de très nombreux traits chez les individus captifs. Parmi les changements observés chez les individus domestiques, on peut compter des modifications de leur comportement (Fleming et Einum, 1997; Huntingford, 2004; Moyle, 1969; Tymchuk *et al.*, 2007), une diminution de leur sensibilité à l'environnement d'élevage qui peut se traduire par une diminution de leur réponse au stress (Solberg *et al.*, 2013a), une baisse de leur réponse anti-prédateurs (Huntingford, 2004; Solberg *et al.*, 2013a; Tymchuk *et al.*, 2006), une augmentation de leur agressivité (Fleming et Einum, 1997; Huntingford, 2004), une diminution de leur résistance aux pathogènes (Glover *et al.*, 2004; Lamaze *et al.*, 2014), ou encore des modifications profondes au niveau des mécanismes cellulaires comme la régulation de l'expression génétique (Solberg *et al.*, 2012) ou la transcription (Sauvage *et al.*, 2010). Au niveau phénotypique, les individus domestiques et sauvages peuvent alors présenter de nombreuses différences. Notamment, la divergence morphologique entre les individus d'élevage et leurs conspécifiques sauvages a souvent été étudiée (Fleming et Gross, 1989; Pulcini *et al.*, 2013; Taylor, 1986). Si elle est souvent attribuée à des effets environnementaux (von Cramon-Taubadel *et al.*, 2005; Fleming *et al.*, 1994; Swain *et al.*, 1991), elle a néanmoins une base génétique (Fleming et Einum, 1997; Pulcini *et al.*, 2013; Taylor et McPhail, 1985) et peut donc être influencée durablement par le phénomène de domestication. Un autre trait largement modifié suite à la domestication est la croissance. En effet, il est courant que les individus domestiques soient sélectionnés artificiellement pour avoir une maturation sexuelle tardive et une croissance importante et rapide (Thorpe, 2004). Ils présentent donc souvent une croissance plus importante que les sauvages (Fleming et Einum, 1997; Jonsson et Jonsson, 2006;

McGinnity *et al.*, 1997, 2003; Reinbold *et al.*, 2009; Solberg *et al.*, 2013a, 2013b; Tymchuk *et al.*, 2006) car ils semblent allouer leur énergie à la croissance et à l'accumulation de gras plutôt qu'à d'autres fonctions biologiques (Crespel *et al.*, 2013a) comme l'immunité (Lamaze *et al.*, 2014; Mangel et Stamps, 2001). Ce dernier compromis entre la croissance et l'immunité se ferait au détriment des capacités immunitaires, ce qui pourrait être également une conséquence potentiellement importante de la domestication. Les traitements contre les pathogènes dans les élevages pourraient également aboutir à un relâchement de la pression de sélection amplifiant cette diminution des capacités immunitaires (Lamaze *et al.*, 2014).

Chaque population subit des pressions de sélection propres à son environnement et développe dans la mesure du possible des traits qui lui procurent des avantages dans cet environnement particulier, indépendamment des conséquences sur l'aptitude phénotypique que ces traits auraient dans un autre habitat (Kawecki et Ebert, 2004). Les traits phénotypiques potentiellement altérés par la domestication sont donc d'une importance capitale pour l'aptitude phénotypique des individus. La morphologie peut par exemple influencer directement ou indirectement des traits liés à la performance et donc ultimement l'aptitude phénotypique (Arnold, 1983; Kingsolver et Huey, 2003), par exemple à travers ses effets sur l'utilisation des ressources (Schluter, 1995), la performance de nage (Blake, 2004; Taylor et McPhail, 1985) ou encore les rapports de dominance qui s'expriment à travers les comportements agonistiques (Holtby *et al.*, 1993). La croissance est quant à elle associée à la survie (Beamish *et al.*, 2004; Hutchings, 1993) et à la reproduction (Morita et Takashima, 1998; Roff, 1983) et une modification des taux de croissance des individus domestiques est susceptible de diminuer leur aptitude phénotypique lorsqu'ils sont relâchés dans le milieu naturel (Tymchuk *et al.*, 2006). Enfin, la défense contre les pathogènes, et notamment les parasites, est également un déterminant de la survie (Pennycuick, 1971) et du succès reproducteur (Bagamian *et al.*, 2004; Barber, 2002; Neff et Cargnelli, 2004; Tierney *et al.*, 1996). Il faut donc prêter une attention particulière à ces problématiques dans le cadre d'introductions d'individus issus de l'élevage dans les populations sauvages. La préservation de l'aptitude phénotypique des individus doit en effet faire partie des objectifs des programmes de supplémentation, la domestication pouvant en effet aboutir à des phénotypes peu performants dans le milieu naturel (Araki *et al.*, 2008; Bellinger *et al.*, 2014; Christie *et al.*, 2012a). La mauvaise aptitude des individus domestiques

pourrait alors affecter l'aptitude globale de la population pour les générations suivantes (Araki *et al.*, 2009). La reproduction d'individus sauvages avec des individus domestiqués pourrait par exemple mener à une progéniture moins nombreuse ou ayant une aptitude réduite (Araki *et al.*, 2007a, 2009; Christie *et al.*, 2014).

Outre son influence sur de nombreux traits individuels, la domestication peut également affecter des paramètres populationnels. En effet, le fait de conserver des populations captives est par exemple souvent lié à une diminution de leur diversité génétique que ce soit à des loci neutres (Skaala *et al.*, 2004) ou pour des traits quantitatifs (Besnier *et al.*, 2015; Solberg *et al.*, 2013a). Cette conséquence des conditions d'élevage doit être prise en compte dans le cadre d'une introduction d'individus domestiques dans les populations sauvages. Une priorité lors des programmes de supplémentation doit en effet être de préserver la diversité génétique de la population ciblée. Elle devra être au moins maintenue suite à l'ajout d'individus extérieurs à la population, bien que la perte de diversité génétique en élevage soit courante (Christie *et al.*, 2012b; Skaala *et al.*, 2004). Souvent, dans le cadre d'introductions intraspécifiques intentionnelles, une perte de variation génétique est observée (Olden *et al.*, 2004). Ainsi, dans un cadre de supplémentation de populations, les effets de la domestication ne doivent pas être sous-estimés et doivent être pris en compte tant au niveau individuel que populationnel.

La pression de pêche et la production aquacole s'intensifient rapidement dans le monde et la proportion de populations surexploitées est en constante augmentation (FAO, 2018). Les populations de poissons, et particulièrement les salmonidés, subissent d'une manière générale des déclins importants dans le monde (Myers et Worm, 2003), notamment en Amérique du Nord (Moyle et al. 2017; Post *et al.*, 2002). Les introductions de poissons d'élevage sont une méthode courante pour pallier ces diminutions (Tringali et Bert, 1998). Dans le contexte spécifique de la conservation des poissons, on ne parle pas d'introductions mais d'ensemencements. Les ensemencements sont devenus une méthode courante pour soutenir les populations menacées ou exploitées. Ils peuvent concerner tant les espèces indigènes qu'introduites. Il existe

également des ensemencements qui ne sont pas destinés à soutenir des populations, mais à satisfaire une demande de pêche dans un lieu donné. Dans ce genre de situation, les ensemencements peuvent mettre en danger les populations locales si des espèces non indigènes sont introduites dans un but récréatif (Hickley et Chare, 2004). Les ensemencements peuvent donc être de différents types en fonction de l'objectif qu'ils se proposent de remplir.

L'origine des poissons ensemencés et la méthode d'élevage sont des composantes importantes des ensemencements et peuvent varier selon les situations (Tableau 1.1, Araki *et al.*, 2008). Ces deux paramètres sont pourtant déterminants quant aux impacts des ensemencements sur les populations supplémentées. Le fait d'utiliser des individus sauvages comme reproducteurs en élevage puis de relâcher leur progéniture à la génération suivante a pour avantage principal une limitation maximale du phénomène de domestication. Cette dernière ne peut toutefois pas être évitée totalement (Lorenzen *et al.*, 2012), même avec une seule génération d'élevage (Christie *et al.*, 2012a; Ford, 2002; Fraser *et al.*, 2018; Waples, 1999). Une baisse d'aptitude phénotypique reste possible même en utilisant des individus locaux (Ford, 2002), mais elle devrait être minimisée par rapport à l'utilisation d'individus non locaux, si bien que l'utilisation de poissons locaux devrait être préférée lorsque c'est possible (Araki *et al.*, 2008; Leonard *et al.*, 2013). L'utilisation d'individus non locaux quant à elle est moins coûteuse et potentiellement moins compliquée à mettre en place. Cependant, elle est susceptible d'altérer la structure génétique des populations sauvages (c'est-à-dire modifier la variation génétique, estomper les adaptations locales, Marie *et al.*, 2010; Valiquette *et al.*, 2014). Les ensemencements font donc partie des activités humaines qui sont susceptibles de modifier la composition et la structure génétique des populations supplémentées et donc d'affecter la diversité génétique de ces dernières (ex. Marie *et al.*, 2010). C'est pourquoi il est donc important de surveiller et caractériser les conséquences de ces programmes, particulièrement lorsqu'ils s'inscrivent sur le long terme (ex. Hansen *et al.*, 2009).

Quelle que soit la méthode de production des individus ensemencés, la domestication doit être prise en compte en raison de ses effets rapides (Christie *et al.*, 2012a; Fraser *et al.*, 2018) et difficilement évitables étant donné qu'ils peuvent résulter d'une sélection involontaire (Fleming et Gross, 1989; Hutchings et Fraser, 2008; Uusi-Heikkilä *et al.*, 2017; Wilke *et al.*, 2015). Les changements génétiques dus à la domestication peuvent varier selon la méthode d'élevage employée et le temps passé par les poissons dans l'environnement artificiel (Araki *et al.*, 2008). En effet, le milieu d'élevage mène à un relâchement de la pression de sélection qui peut compromettre le succès des ensemencements si les individus sont mal adaptés ou ont accumulé des mutations délétères (Lynch et O'Hely, 2001). Un autre inconvénient inhérent à la méthode des ensemencements est que le fait de faire se reproduire des poissons en captivité augmente artificiellement beaucoup le nombre de leurs descendants qui vont survivre par rapport aux conspécifiques sauvages. Qu'il s'agisse d'individus locaux ou non, une partie de la population aura été grandement favorisée par rapport aux autres, ce qui démultiplie la variance de succès reproducteur interindividuel. Or ce paramètre est très important pour la taille effective et cette augmentation de la variance de succès reproducteur peut directement mener à une diminution de la taille effective (Ryman et Laikre, 1991). Il n'existe donc pas de méthode qui permette d'augmenter la taille des populations naturelles sans contrepartie, bien que l'utilisation d'individus locaux ayant passé peu de temps en captivité soit préférable (Araki *et al.*, 2008). Parfois, il est même possible qu'un programme de supplémentation d'une population en déclin ait finalement plus d'effets délétères que d'effets positifs (Reisenbichler et Rubin, 1999). Dans le cas où l'utilisation d'individus locaux ne serait pas possible (ex. ensemencement de repeuplement, introduction d'une nouvelle population…), les individus reproducteurs seront issus d'une souche du milieu d'élevage et ils pourront alors subir les effets de la domestication depuis un nombre variable de générations.

Introgression génétique

Un risque intrinsèque à la supplémentation des populations sauvages avec des individus d'élevage est l'hybridation entre domestiques et sauvages. En effet, les pratiques d'élevage mènent souvent à une homogénéisation génétique chez de nombreuses espèces de poissons (Le Cam *et al.*, 2015; Lamaze *et al.*, 2012; Marie *et al.*, 2010; Olden *et al.*, 2004; Valiquette *et al.*, 2014) et le bagage génétique des individus domestiques diverge alors rapidement de celui de leurs conspécifiques sauvages, les rendant aisément différenciables (ex. Marie *et al.*, 2010; Ozerov *et al.*, 2016). L'hybridation est définie comme la reproduction entre des individus appartenant à deux populations ou espèces distinctes. Dans certaines circonstances, ce processus peut mener au déclin, voire à la disparition de populations ou même d'espèces (Evans et Willox, 1991). En effet, il peut entraîner la « dilution » des caractéristiques génétiques des deux populations (c'est-à-dire si une espèce rare s'hybride avec une espèce commune qui en est proche, l'espèce commune peut, à terme, « assimiler » l'espèce rare), ou la réduction du potentiel reproducteur des individus qui donnent des descendants hybrides stériles. Si toutefois la descendance qui suit l'hybridation est fertile et que la progéniture continue de se reproduire, d'une part entre hybrides et d'autre part entre hybrides et individus issus des souches parentales, un flux de gènes est établi entre les populations qui s'hybrident et on parle alors d'introgression génétique (Rhymer et Simberloff, 1996). L'intégrité de la population d'origine peut alors être menacée par un phénomène de pollution génétique si des allèles maladaptatifs sont introduits (Iacolina *et al*., 2019, Puigcerver *et al*., 2014). Bien que généralement perçus comme nuisibles (Iacolina *et al*., 2019), les effets de l'introgression ne sont pas toujours négatifs. Des phénomènes de résistance à certains parasites ont par exemple été observés dans des zones hybrides entre deux espèces de souris (Moulia et al., 1995). Dans certaines circonstances, l'introgression pourrait même s'avérer bénéfiques pour certains traits tout en étant délétères pour d'autres, ayant donc un effet global nuancé sur l'aptitude phénotypique des individus tel que montré chez le sanglier (*Sus scrofa*, Fulgione *et al*., 2016). Dans le cadre de mon projet, je me suis intéressée uniquement à l'introgression génétique intraspécifique.

Table 1.1 Différents types d'ensemencements en fonction du type d'élevage. Adapté de Araki *et al.* 2008.

	Reproducteurs d'origine non locale	Reproducteurs d'origine locale
Reproducteurs viennent tous du milieu d'élevage	<u>Reproducteurs d'origine non locale séparés :</u> tous les reproducteurs sont issus de l'aquaculture et sont potentiellement domestiqués depuis de nombreuses générations	<u>Reproducteurs d'origine locale séparés :</u> les reproducteurs sont originaires de l'endroit où on veut les ensemencer, eux ou leur progéniture, mais ils viennent néanmoins tous de l'aquaculture
Reproducteurs mélangés entre milieu d'élevage et sauvage		<u>Reproducteurs d'origine locale non séparés :</u> tous les reproducteurs viennent de l'endroit où on veut les ensemencer eux ou leur progéniture, une partie d'entre eux vient de l'aquaculture et une partie d'entre eux est sauvage

Dans le cadre des ensemencements, les effets de l'introgression génétique pourraient s'avérer délétères, en raison d'une aptitude phénotypique réduite des individus ensemencés suite à la domestication (Araki *et al.*, 2008; Christie *et al.*, 2014), ce qui diminuerait le rendement de la population (Reisenbichler et Rubin, 1999). En effet, la domestication influençant des caractéristiques phénotypiques et immunitaires importantes pour l'aptitude phénotypique (ex. morphologie, croissance, défense contre les parasites; voir la section « *Ensemencements et domestication* »), l'introgression de gènes domestiques pourrait avoir des effets néfastes sur la performance globale des populations supplémentées (McGinnity *et al.*, 1997, 2003). De plus, la création de « zones hybrides » peut avoir des conséquences variables et difficilement prévisibles

sur certains paramètres populationnels tels que les relations hôtes-parasites (Theodosopoulos *et al.*, 2019).

Un autre mécanisme par lequel l'introgression génétique peut avoir des effets néfastes sur les populations sauvages est la perte d'adaptations locales en raison de l'introduction d'allèles exogènes (Naish *et al.*, 2008; Taylor, 1991). En effet, les adaptations locales sont courantes et jouent un rôle important dans l'aptitude phénotypique des salmonidés (Fraser *et al.*, 2011; Taylor, 1991). Cependant, l'hybridation intraspécifique peut diluer ces adaptations locales lorsque des individus d'une population exogène sont introduits (Olden *et al.*, 2004). La perte de ces adaptations locales pourrait alors avoir des effets délétères sur les populations supplémentées (Bourret *et al.*, 2011; Tymchuk *et al.*, 2007, mais voir Fraser, 2008 pour un exemple d'introgression qui a des effets contrastés sur l'adaptation locale). Enfin, l'introgression génétique peut affecter les populations en raison de la dépression hybride. Cette dernière, aussi appelée dépression d'exogamie, est un phénomène se produisant lorsque des individus génétiquement trop éloignés l'un de l'autre se reproduisent. Elle est donc susceptible de se produire lorsque des individus domestiques et sauvages aux patrimoines génétiques différents cohabitent et se reproduisent. Dans cette situation, il est possible qu'il y ait une rupture de complexes de gènes coadaptés (c'est-à-dire des gènes qui ont évolué ensemble dans un même environnement et qui fonctionnent en synergie) et les hybrides ne sont donc adaptés à aucun des deux environnements des populations mères, ce qui génère une baisse de leur aptitude phénotypique (Frankham *et al.*, 2004).

L'introgression peut donc affecter l'intégrité génétique des populations sauvages (Allendorf *et al.*, 2001; Glover *et al.*, 2012) et elle peut également fortement ralentir ou empêcher la purge d'allèles délétères par la sélection naturelle, ce qui peut causer un déclin de l'aptitude de la population sur le long terme (Fleming et Einum, 1997). Par ailleurs, les effets de l'introgression peuvent également s'estomper d'eux-mêmes après quelques générations (Létourneau *et al.*, 2018; Valiquette *et al.*, 2014; White *et al.*, 2018), notamment si la sélection contre les individus

introgressés est forte (Hutchings et Fraser, 2008) ou s'il y a peu d'ensemencements et donc qu'il y a potentiellement peu d'introgression (Tymchuk *et al.*, 2006). À l'inverse, dans certaines situations, l'introgression peut se répandre malgré une aptitude réduite chez les hybrides, par exemple dans le cas où les gènes introgressés favorisent leurs capacités de dispersion ce qui leur donne l'opportunité de coloniser des niches qui étaient vacantes et de s'y reproduire entre eux puis de coloniser les autres habitats grâce à leur dispersion importante (Lowe *et al.*, 2015). Les effets de l'introgression peuvent en effet dépendre de la structure génétique de la population considérée et varier d'une population à l'autre d'une façon difficile à prévoir (Glover *et al.*, 2012; Leitwein *et al.*, 2018; Normandeau *et al.*, 2009). Les niveaux d'introgression peuvent ainsi être très différents selon les endroits (Hansen *et al.*, 2009), allant même jusqu'à des variations de presque zéro à la quasi-totalité des poissons introgressés, notamment en fonction des conditions environnementales (Marie *et al.*, 2012; Yau et Taylor, 2013) et/ou de la force de la sélection qui s'applique contre les individus introgressés (Hansen, 2002). Cependant, d'une manière générale, les conséquences génétiques de l'hybridation entre les individus domestiqués et les populations sauvages sont imprévisibles (Bougas *et al.*, 2010; Granier *et al.*, 2011) et sont souvent délétères pour ces dernières (Hindar *et al.*, 1991; Hutchings et Fraser, 2008), principalement à cause du phénomène de domestication (ex. Araki *et al.*, 2007a; McGinnity *et al.*, 2003; Tymchuk et Devlin, 2005). L'introgression génétique est ainsi souvent considérée comme une menace importante pour les populations sauvages, voire pour les espèces, dans le cadre d'introgression interspécifique (Epifanio et Philipp, 2000; Frankham *et al.*, 2002; Rhymer et Simberloff, 1996). Dans cette étude, j'utiliserai différents termes pour me référer à l'origine des individus et à leur bagage génétique. Leur définition est donnée dans le Tableau 1.2.

Table 1.2 Définition des termes associés à l'origine et au bagage génétique des individus considérés dans cette étude.

Terme	Définition
Sauvage	Un individu est considéré comme sauvage lorsqu'il n'a aucune ascendance détectable d'origine domestique.
Domestique	Un individu est considéré comme domestique lorsqu'il est né en aquaculture, et ce quel que soit le nombre de générations que ses ancêtres y ont passé avant lui.
Hybride	Un individu est considéré comme hybride (c'est-à-dire introgressé) lorsqu'il possède un bagage génétique mixte entre la souche sauvage et la souche domestique, quelles que soient les proportions de son génome qui viennent de l'une ou l'autre des deux souches.

Les problèmes liés à l'introgression génétique ont déjà été observés chez de nombreux taxa (ex. Fitzpatrick *et al.*, 2010; Puigcerver *et al.*, 2014; Rhymer et Simberloff, 1996; Verardi *et al.*, 2006), mais les études sur l'introgression sont particulièrement abondantes chez les poissons en raison de ses nombreuses conséquences sur des populations exploitées. Les salmonidés, compte tenu de leur importance économique, ont été particulièrement étudiés. Malgré ces travaux, les informations sur les effets de l'introgression restent partielles et peuvent parfois être contradictoires (ex. Collis *et al.*, 2001; Osterback *et al.*, 2014). En particulier, les effets de l'introgression génétique sur l'aptitude phénotypique représentent un enjeu important, tant économique que scientifique, et de nouvelles études sur l'influence des ensemencements et de l'introgression génétique sur les populations naturelles aideraient à mieux comprendre les impacts des programmes d'ensemencements et à mieux anticiper leurs conséquences.

Les études précédentes, menées en partie dans le même système d'étude que celui de mes travaux, ont permis de mieux comprendre les facteurs qui modulent l'introgression génétique

chez l'omble de fontaine (*Salvelinus fontinalis*) (Lamaze *et al.*, 2012, 2014; Marie *et al.*, 2010, 2012). Mon étude est donc complémentaire aux recherches déjà menées, car mon travail consiste à déterminer quelles sont les conséquences de l'introgression.

Objectifs

L'objectif général de ma thèse est de mieux caractériser les impacts des ensemencements d'individus domestiques et de l'introgression génétique dans des populations sauvages de salmonidés. Je m'intéresse pour cela aux conséquences des ensemencements et de l'introgression tant au niveau individuel que populationnel. Afin de répondre à cet objectif, j'utilise comme modèle d'étude l'omble de fontaine, un salmonidé massivement ensemencé dans le Sud du Québec. Ma thèse est divisée en trois chapitres dont les objectifs spécifiques sont respectivement :

1. Identifier les effets de l'origine génétique des individus (domestiques, sauvages ou hybrides) sur leur phénotype, plus spécifiquement ici leur morphologie, leur croissance et leur taille à chaque âge. À titre complémentaire, déterminer si l'origine génétique influence la niche et/ou le niveau trophique des individus.

2. Évaluer l'impact des ensemencements et de l'introgression génétique sur les relations hôtes-parasites, tant au niveau individuel (ex. statut infectieux, intensité de l'infection) que populationnel (ex. prévalence, diversité de la faune parasitaire).

3. Déterminer les impacts des ensemencements et de leur intensité sur les tailles effectives des populations supplémentées.

Méthodes

Modèle d'étude et contexte des ensemencements dans le système

Mon travail se place dans le contexte d'ensemencements de mise en valeur au Québec. La pêche sportive et récréative est une activité économiquement très importante au Canada. En 2015, c'est plus de 2,5 milliards de dollars de dépenses directes qui lui étaient liés, dont plus de 498 millions de dollars au Québec. Cette activité génère également des milliers d'emplois. Toujours en 2015, plus de 3,2 millions de pêcheurs adultes ont participé à des activités de pêche récréative, dont plus de 652 000 résidents du Québec. Uniquement dans cette province, ce sont plus de 43 millions de poissons qui ont été pêchés cette année-là (Pêches et Océans Canada, 2015). Les populations naturelles de poissons ont décliné jusqu'à des points critiques au Canada sous la pression de la pêche récréative (Post *et al.*, 2002). Les ensemencements sont alors devenus une méthode répandue pour que les populations puissent supporter l'effort de pêche. Le poisson le plus populaire et le plus pêché au Québec est l'omble de fontaine, un salmonidé commun dans les lacs et les cours d'eau. En 2012 au Québec, la production totale d'ombles de fontaine à des fins d'ensemencements était d'environ 670 tonnes pour l'année, la majeure partie de cette production venant des pisciculture privées. Cela représente environ 5 millions d'ombles de fontaine potentiellement pêchables (soit au-dessus de 150 g) (Ministère du Développement durable, de l'Environnement, de la Faune et des Parcs, 2013).

L'omble de fontaine est un membre de la famille des *Salmonidae*. Il est endémique de l'Amérique du Nord (Bernatchez et Giroux, 2000) et peut présenter une variabilité très importante aux niveaux génétique, phénotypique et moléculaire d'une population à l'autre, et ce même à faible échelle spatiale (Angers *et al.*, 1995; Kazyak *et al.*, 2015). De nombreuses populations de lacs sont différenciées génétiquement les unes des autres (ex. Kazyak *et al.*,

2015, 2016), ce qui fait de cette espèce un excellent modèle de génétique des populations et pour les études sur les adaptations locales.

D'une manière générale, peu d'études ont pu évaluer les effets directs de l'introgression sur l'aptitude sur plusieurs générations (mais voir McGinnity *et al.*, 2003; Tymchuk *et al.*, 2007). De plus, Tymchuk *et al.* (2007) ont montré que les effets délétères de l'introgression génétique sur la survie en situation de prédation n'apparaissent qu'au bout de trois générations d'introgression, d'où l'intérêt de considérer un nombre important de générations dans ce type de travaux. Le système d'étude des ensemencements d'ombles de fontaine au Québec est ainsi particulièrement intéressant pour une analyse des conséquences de l'introgression génétique. En effet, les historiques d'ensemencements des réserves fauniques sont bien documentés et les ensemencements sont pratiqués depuis de nombreuses années, ce qui permet de comprendre comment l'introgression a pu agir à long terme sur les populations.

Données disponibles

Trois réserves fauniques (Mastigouche, Portneuf et Saint-Maurice) situées dans le Sud du Québec (Figure 1.1) ont été choisies pour la collecte d'échantillons. Deux d'entre elles (Portneuf et Mastigouche) avaient déjà été échantillonnées dans le cadre d'un précédent projet en 2007 et 2008. Ces précédents travaux ont permis de mettre en évidence la présence de l'introgression d'allèles domestiques, ainsi qu'une homogénéisation génétique globale des populations ensemencées (Marie *et al*., 2010). Ils ont également mis en évidence le fait que les facteurs environnementaux modulent les niveaux d'introgression (Marie et al., 2012), permettant ainsi de mieux comprendre les facteurs qui causent et influencent l'introgression génétique. Ces réserves consignent l'historique d'ensemencements de leurs lacs depuis les années 60, ce qui a permis d'identifier des lacs avec des niveaux d'ensemencements très variés. En 2014, des

échantillonnages préliminaires ont eu lieu et ont été suivis de deux années d'échantillonnage (2015 et 2016) pendant mon doctorat.

Pendant l'été, soit avant les ensemencements de l'année d'échantillonnage, les poissons ont été capturés à l'aide de filets maillants. Chaque individu a ensuite été mesuré, pesé, photographié, inspecté pour la présence de parasites externes puis disséqué pour contrôler la présence de parasites internes et pour déterminer le sexe. Le corps des poissons a été congelé afin d'en extraire ultérieurement les otolithes. La chair de certains individus a été prélevée pour un dosage ultérieur d'isotopes stables. La nageoire adipeuse a été prélevée afin d'en extraire l'ADN en laboratoire par une méthode d'extraction saline (Aljanabi et Martinez, 1997).

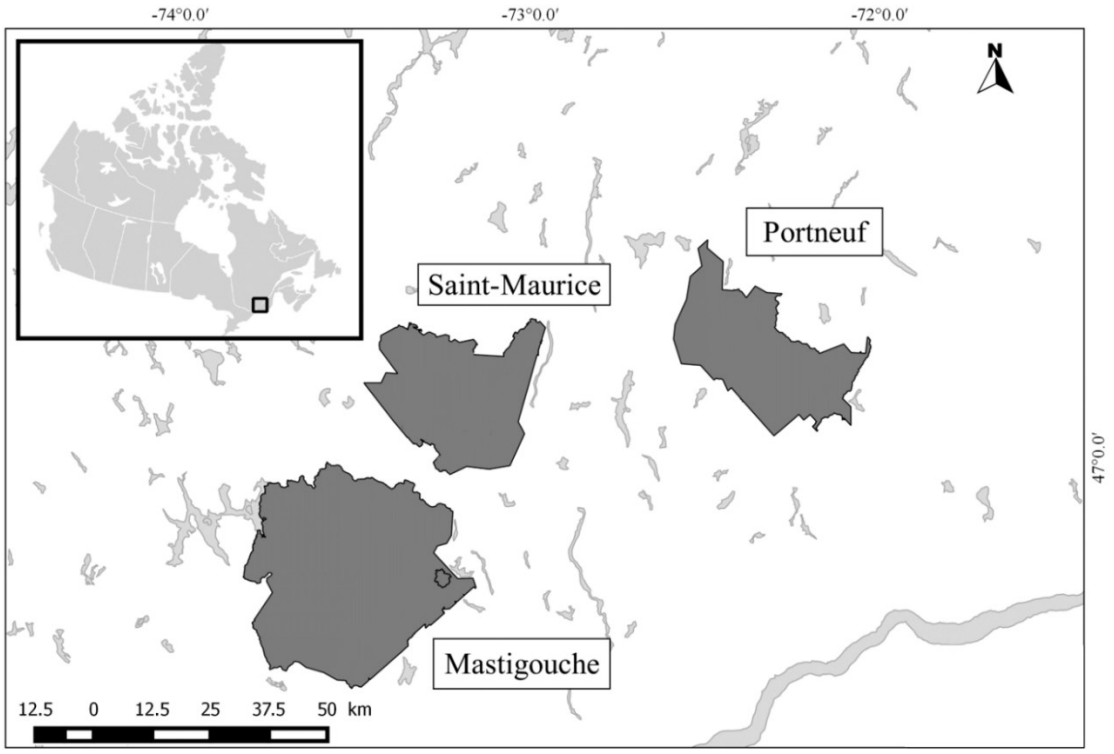

Figure 1.1 Carte du site d'étude.

Les zones représentées en gris foncé représentent les réserves fauniques échantillonnées.

Une fois l'ADN extrait, chaque individu a été génotypé à 20 loci microsatellites. Ces marqueurs génétiques neutres ont été largement utilisés dans un contexte de conservation, notamment dans le cadre de la gestion des pêcheries (Moran, 2002; Wright et Bentzen, 1994). Ils ont également déjà fait leurs preuves dans le contexte de la détection de l'introgression génétique (Hansen, 2002; Hansen et Mensberg, 2009; Marie *et al.*, 2010, 2012). Ces données ont donc servi à assigner chaque individu à une origine génétique grâce au logiciel STRUCTURE (Pritchard *et al.*, 2000) dont l'efficacité a déjà été montrée (Marie *et al.*, 2011), notamment lorsqu'il est utilisé en combinaison avec des marqueurs microsatellites (Sanz *et al.*, 2009).

L'ensemble des données génétiques est ainsi issu d'un total de 42 lacs échantillonnés entre 2007 et 2016, dont 12 ont été échantillonnés au cours des deux périodes de captures (2007-2008 et 2014-2016). Au total, 3361 poissons ont été capturés et génotypés. Plus précisément, dans le cadre des analyses de croissance et de morphologie, un sous-échantillon de 12 lacs a été utilisé et 4 lacs ont quant à eux été utilisés pour les analyses d'isotopes stables (Chapitre 2). Pour les analyses de parasitisme, 28 lacs ont été considérés (Chapitre 3). Enfin, pour les analyses de taille effective, la totalité des 54 populations (42 lacs dont 12 ont été échantillonnés sur les 2 périodes de captures) a été utilisée (Chapitre 4).

CHAPITRE 2
PHÉNOTYPE

Description de l'article et contribution

La domestication affecte fortement le phénotype des individus d'élevage, notamment la morphologie et la croissance, en raison de la sélection artificielle et d'un environnement drastiquement différent de l'environnement naturel. Cependant, peu d'informations sont disponibles sur le maintien de ces différences et l'importance de l'origine génétique lorsque les individus sont dans le même environnement (ex. en cas d'ensemencements). Le but de cet article était donc d'évaluer comment la morphologie, la croissance et la taille à chaque âge sont influencées par l'origine génétique des individus. De façon complémentaire, la niche et le niveau trophique ont également été analysées en lien avec l'origine génétique des poissons. Les résultats indiquent un effet modéré et environnement-dépendant de l'origine génétique sur le phénotype. De plus, les individus domestiques occupent des niches trophiques plus benthiques et se nourrissent à des niveaux trophiques plus élevés que les sauvages et hybrides, suggérant que les différences phénotypiques pourraient être expliquées par des habitudes alimentaires différentes selon l'origine génétique. La variation phénotypique observée restait principalement liée à l'identité du lac, indiquant que l'environnement est le principal facteur explicatif de la morphologie, de la croissance et de la taille dans les populations ensemencées.

Pour cet article, j'ai participé à la collecte des données (2015-2016), et j'ai réalisé le travail de laboratoire. Je tiens à remercier Raphaëlle Dubois et Nicolas Bousquet pour leur aide concernant le travail de terrain, ainsi qu'Anne-Lise Fortin pour son travail de préparation des otolithes. L'élaboration des idées et l'interprétation des résultats s'est faite en collaboration avec Dany Garant. J'ai analysé les données et rédigé le manuscrit. Dany Garant a révisé plusieurs versions du manuscrit. Louis Bernatchez et Pascal Sirois ont révisé le manuscrit.

Effects of genetic origin on phenotype in lakes stocked with domestic fish

Soumis à *Ecological Applications*.

Philippine Gossieaux, Émilie Lavoie, Pascal Sirois, Isabel Thibault, Louis Bernatchez, Dany Garant

Abstract

Phenotypic changes due to human activities are occurring at a far greater speed than those originating from natural causes in animal populations. For instance, phenotypic divergence among individuals may arise in populations supplemented with farm-reared fish that are known to display different phenotypes from those of wild individuals because of domestication. However, little is known about how these phenotypic differences are maintained when domestic and wild individuals face the same environment and hybridize, as it is the case after supplementation. In this study, we assessed the effect of genetic origin of individuals on phenotypic trait divergence (morphology, growth and size-at-age) in stocked populations of Brook Trout (*Salvelinus fontinalis*). We also evaluated whether genetic origin influences habitat use by documenting trophic niche and/or level using stable isotope analyses. We found significant effects of genetic origin on phenotypic variables with domestic fish generally being more fusiform, larger and having higher growth rates than other individuals. These effects were often in interaction with the lake identity, suggesting that they are mostly population-dependent. Lake identity also explained most of the variation in phenotypic variables, meaning that population-specific attributes were important drivers of morphology and growth. Our result also showed that domestic fish were feeding in more littoral niches and at a higher trophic level than hybrid individuals, suggesting that differences in feeding habits could partially explain phenotypic differences. These results highlight the importance of accounting for the genetic composition of populations when assessing the causes of phenotypic divergence in the wild.

Keywords: Geometric morphometrics; Otoliths; Stable isotopes; Stocking; Salmonids

Introduction

Different ecological contexts can generate phenotypic variability in natural populations, eventually leading to the coexistence of different ecotypes at various geographical scales (Lavin and McPhail 1986, Taylor 1999, O'Reilly and Horn 2004, Marcil et al. 2006, Crispo and Chapman 2010, Perreault-Payette et al. 2017). More locally, differential use of resources or habitat among individuals within a given population can also lead to phenotypic divergence (e.g. Bourke et al. 1997, Douglas et al. 1999, Landry et al. 2007, Hendry et al. 2009, Harrod et al. 2010, Svanbäck and Schluter 2012, Baillie et al. 2016). While the determinants of phenotypic variation and differentiation in a population can be natural (see Landry et al. (2007) for instance), it can also often result from human actions (Hendry et al. 2008). In fact, human activities have been shown to induce phenotypic changes at a far greater speed than natural causes in animal populations (Hendry et al. 2008, Alberti et al. 2017). A common modification induced by human actions is the intentional introduction of exogenous individuals in wild populations, which is frequently performed for conservation or management purposes (Brown and Day 2002, Tallmon et al. 2004, Naish et al. 2008, Laikre et al. 2010, Scribner et al. 2018). These exogenous individuals can be either transferred from other wild populations (Tallmon et al. 2004) or originate from farms where they are bred with the objective of being released in the wild (Laikre et al. 2010).

Farm-raised individuals are affected by domestication, a widespread and mostly unavoidable phenomenon that can occur as a result of active artificial selection, or that can also occur involuntarily (Fleming and Gross 1989, Hutchings and Fraser 2008, Wilke et al. 2015, Uusi-Heikkilä et al. 2017) and in as little as a single generation of captivity (Christie et al. 2012, Fraser et al. 2018). Major consequences of domestication often include a decrease of fitness for domestic individuals when compared to their wild counterparts in natural environments (Araki et al. 2008, Christie et al. 2012, 2014). In some cases, the impacts of domestication could be potentially deleterious enough to strongly decrease the efficiency of animal introduction for

conservation measures (Laikre et al. 2010) because of its effects on survival (McGinnity et al. 1997, 2003, Skaala et al. 2019) or reproductive success (Jonsson and Jonsson 2006, Araki et al. 2009, Christie et al. 2014) of domestic individuals.

Releasing domesticated individuals into wild populations is a widespread action in the context of fisheries management (Brown and Day 2002, Araki and Schmid 2010). In particular, the consequences of domestication have been well studied in salmonids given that they have been massively farmed and stocked around the world for decades (Naish et al. 2008, Hutchings and Fraser 2008, Lorenzen et al. 2012). In these species, morphological traits seem particularly affected by domestication because of the differences between selective pressures in aquaculture and in the wild (Taylor 1986, Swain et al. 1991, Fleming et al. 1994, Fleming and Einum 1997, Jonsson and Jonsson 2006, Pulcini et al. 2013). For instance, environmental conditions such as water temperature (Beacham 1990), population density (Jonsson and Jonsson 2006) or stream velocity (Peres-Neto and Magnan 2004, Jonsson and Jonsson 2006, Samways et al. 2015) during early life have a crucial importance for body shape (Jonsson & Jonsson 2006, 2014) and differ significantly between natural and artificial habitats (Thorpe 2004, Jonsson and Jonsson 2006, Blanchet et al. 2008). Even though morphological divergence can often be explained by phenotypic plasticity (Peres-Neto and Magnan 2004, Jonsson and Jonsson 2006, Samways et al. 2015), it has been showed in some cases that morphological differences between wild and domestic fish have a genetic basis (e.g. Taylor and McPhail 1985, Swain et al. 1991, Fleming and Einum 1997, Pulcini et al. 2013). Furthermore, morphological divergence from the wild phenotype is stronger for domestic strains that have been kept captive during multiple generations compared to strains that have only been recently domesticated (Fleming et al. 1994).

Another key trait strongly impacted by domestication, because it is targeted by artificial selection, is growth. Fish farmers typically aim at producing fast-growing individuals by delaying the age of sexual maturation (Thorpe 2004, Jonsson and Jonsson 2006). Domestic fish thus generally have higher growth rates and size at a given age than their wild counterparts

(Fleming and Einum 1997, McGinnity et al. 1997, 2003, Tymchuk et al. 2006, Reinbold et al. 2009, Solberg et al. 2013b, 2013a) and these differences have been shown to have a genetic basis (Tymchuk and Devlin 2005, Tymchuk et al. 2006, Crespel et al. 2013a, Berejikian et al. 2017). However, the difference in growth rates between domestic and wild individuals seems to be dependent on the context in which they are quantified. Indeed, in natural conditions, wild individuals can display growth rates similar to those of domestic fish (Reisenbichler and McIntyre 1977, Solberg et al. 2013a, 2013b). While it is well acknowledged that hatchery-reared and wild fish often differ in their phenotypes (Fleming and Einum 1997, Jonsson and Jonsson 2006), it is less clear whether these differences are maintained when domestic and wild individuals face the same environment after stocking. Moreover, when domestic and wild fish hybridize, it can be challenging to anticipate the potential impacts of genetic introgression on the morphology and growth of hybrids within populations (Bougas et al. 2010, Granier et al. 2011).

Our goal in this study is thus to determine how the genetic origin of individuals influences morphology, growth and size-at-age in stocked populations. Individuals with a domestic genetic background have hatched and spent their early life in aquaculture and are thus likely to present phenotypic differences compared to wild fish. The effect of these differences on the phenotype of hybrid individuals will then depend on the genetic basis of the measured traits. If phenotype has a strong genetic basis, phenotypic divergence should be strong between individuals with different genetic origins (e.g. Fleming and Einum 1997, Jonsson and Jonsson 2006) and hybrids would be likely to display an intermediate phenotype (e.g. Schluter 1993, 1995, McGinnity et al. 1997, Reinbold et al. 2009, Skaala et al. 2019). Alternatively, if phenotype is mostly shaped by environmental conditions, phenotypic divergence should be small (e.g. Solberg et al. 2013b) and hybrids should have the same phenotype as wild individuals since they shared the same environment since hatching (e.g. McGinnity et al. 1997, Harbicht et al. 2014). As a complementary question, we used stable isotope analyses to assess whether genetic origin influences resource use in terms of habitat and feeding habits, assessed with stable isotopes analyses, since these are two essential determinants of both morphology (Schluter 1993, 1995,

Bourke et al. 1997, Dynes et al. 1999, Bertrand et al. 2008, Harrod et al. 2010, but see Samways et al. 2015, Andersson et al. 2017) and growth (Schluter 1995, Glaz et al. 2012, 2014, Morissette et al. 2018, 2019).

To investigate these questions, we used Brook Trout (*Salvelinus fontinalis*), a very popular salmonid for recreational angling, which has been massively stocked for decades in North America, and notably in Québec, Canada. Previous studies in this region showed that hybridization between domestic and wild fish is common and that stocked lakes present various levels of introgression of domestic genes (Marie et al. 2010, 2012, Gossieaux et al. 2018, 2019, Létourneau et al. 2018, Gossieaux et al. 2019). We thus used data from 12 introgressed populations to determine the extent of phenotypic divergence between individuals that have different genetic origins. Since Brook Trout may display important plasticity for morphological traits (Peres-Neto and Magnan 2004, Kazyak et al. 2015, Samways et al. 2015, Zastavniouk et al. 2017), if an effect of genetic origin is detectable, we predict that it should not be very strong. Also, since domestic individuals are actively selected for higher growth rates (Thorpe 2004, Jonsson and Jonsson 2006), we predict that domestic fish will outgrow wild individuals in the natural environment. This could be a consequence of genetic effects (Tymchuk and Devlin 2005, Reinbold et al. 2009), early life conditions (Jonsson and Jonsson 2014), or a combination of both (Crespel et al. 2012, 2013a, Berejikian et al. 2017). If rearing conditions are an important determinant of growth, we would predict to observe differences due to genetic origin especially in young age classes since it will be closer from the moment domestic fish were stocked and thus from the time they spent and were fed in hatchery. Moreover, growth differences between domestic and wild fish have been shown to decrease as mortality increases (Solberg et al. 2013b) and we thus expect to see weaker effects of domestic origin in older age classes. Considering the crucial effect of environmental conditions on growth and size (Solberg et al. 2013b, Fraser et al. 2018), we also expect to see a strong effect of environment on these variables. Finally, we should also observe differences in trophic level or trophic niche between wild and domestic individuals since domestication can affect a wide range of behaviors, including feeding behavior (Huntingford 2004) and possibly habitat use (Mittelbach et al. 2014).

Methods

Sampling and procedures

We conducted sampling over two time periods (2007-2008 and 2014-2016) in three wildlife reserves (Portneuf [47°10'17.8"N, 72°20'32.7"W], Mastigouche [46°42'45.2"N, 73°25'37.7"W] and Saint-Maurice [47°04'00.0"N, 73°08'28.5"W]) in Québec, Canada (see Gossieaux et al. 2018). Stocking history of lakes in these reserves has been documented since 1964 (provided by the ministère des Forêts, de la Faune et des Parcs, Québec, Canada) and stocking intensity ranged from lakes that were massively stocked for decades to others that were not stocked for years or were never stocked. In order to stock its lakes, Portneuf reserve uses domestic fish from the Jacques-Cartier hatchery, a facility that kept fish in captivity from multiple generations. Mastigouche and Saint-Maurice reserves stock their lakes with hatchery-reared individuals from Lac-des-Écorces (a governmental facility) and Saint-Alexis-des-Monts hatcheries, which cross domestic and wild fish from Lake Bourassa (located in the Mastigouche reserve) to obtain hybrid strains. Fish are mostly stocked at very early life stages, such as fry.

For the phenotype analyses, we used samples from fish captured with gill nets in 12 lakes (n = 550 fish, Table S2.A1) that are part of a larger study in the three wildlife reserves (see Gossieaux et al. 2019). For the stable isotopes analyses, we used data from four lakes in the same system (n = 438). We sampled fish before the annual stocking events to avoid capturing recently stocked individuals. Therefore, all captured individuals spent at least between 10 and 12 months in the lakes. We euthanized fish with clove oil immediately after each capture. Each individual was then measured (total length, ± 1 mm) and weighed (± 1 g), allowing us to estimate body condition using the Fulton index ($K = mass/length^3$, Cone 1989). For 10 out of 12 populations used in phenotype analyses, individuals were also sexed by observation of gonads during a dissection. Adipose fin of each fish was collected and preserved in 95% ethanol for later DNA

extraction. Moreover, we obtained tissue samples from hatcheries (Jacques Cartier, n=53, Saint-Alexis des Monts, n=80, Lac des Écorces, n=40) and from lake Bourassa (n=40). All protocols and procedures employed were reviewed and approved by the ministère des Forêts, de la Faune et des Parcs (Québec, Canada) (see Gossieaux et al. 2018, 2019).

Genetic analyses

We used adipose fins to extract DNA and genotype each fish at 20 microsatellite loci following the protocols described in Gossieaux et al. (2018). We then determined the genetic origin of each fish using the software Structure v. 2.3.4 (Pritchard et al. 2000) following the assignment method and parameters described in (Gossieaux et al. 2018, 2019). Each fish was then attributed a q-value ranging from 0 to 1, respectively designating pure wild and pure domestic individuals.

Morphometrics data

Quickly after capture, each individual was photographed on the same side on a soft surface to minimize deformation. We chose 18 landmarks (Fig. S2.A1) for every selected individual with the software tpsDig v.2.31 (Rohlf 2005). After digitization, we applied a generalized Procrustes analysis (Rohlf 1999) to superimpose landmark configurations (n = 457). This step removes the variation in landmark configurations due to scale, orientation and location. The resulting transformed landmark coordinates (Procrustes coordinates) can be used as response variable in further statistical analyses as their variation is only attributable to differences in shape between individuals (Webster and Sheets 2010).

To make sure that measurement error was negligible, we digitized a second time 50 randomly selected individuals and estimated the repeatability of each landmark placement using the "rptR" R package (Stoffel et al. 2017). We also used a Procrustes ANOVA to quantify measurement error, which is estimated by an interaction between digitization identity and individual identity (Klingenberg and McIntyre 1998).

Growth data

We estimated individual growth parameters using otoliths. Right and left sagittae were extracted for all individuals, mounted on microscope slides in thermoplastic glue, polished and photographed using a microscope (Panfili et al. 2002). We analyzed photos using the software ImageJ v. 4.51 j8 (Abràmoff et al. 2004). We determined the age of each fish counting annuli and measured transversal width, dorsal radius (DR, µm) and annual increments width along DR (µm) (Fig. S2.A.2). Each photograph was read at least twice by the same reader with over three months between the two readings (Panfili et al. 2002). Depending on otolith quality and on the observer's confidence, a score of 'confidence' was assigned to each reading. This score ranged from 1 to 4 with 1 being very unsure and 4 very confident in the reading (Stevenson and Campana 1992). When there was a mismatch of age estimation between the first two readings, a verification or third reading was performed by the same reader. For each individual, we used the reading confidence scores to decide which reading was kept for further analysis (see Fig. S2.A3 for more details on readings and verifications). We excluded individuals for which otoliths were too damaged and thus kept a total of 487 fish for further analyses (10.8% of rejection)..

Trophic niche and trophic level

Stable isotopes, and more specifically carbon and nitrogen ratios, are widely used to evaluate both trophic niche and trophic level, notably in freshwater ecosystems (Post 2002). Nitrogen ratios ($\delta^{15}N$) are representative of the trophic position with higher scores reflecting a higher trophic level (i.e. more predatory individuals, Minagawa and Wada 1984, Vander Zanden et al. 1997). Carbon ratios ($\delta^{13}C$) are, for their part, used to evaluate trophic niche with low ratios indicating that individuals feed in pelagic environments based on autochthonous production and high ratios being the sign that individuals feed in the littoral zone more enriched by allochthonous subsidies(France 1995, Glaz et al. 2012).

Three lakes of Portneuf reserve and one of Mastigouche reserve (n = 438 fish) were selected for collection of stable isotopes samples between 2007 and 2014 (Table S2.A1). A sample of dorsal muscle without skin and bone was collected for each individual and frozen immediately after capture. We dried muscle samples at 60°C for 48 hours and grinded them to obtain a fine powder that we then encapsulated to obtain samples of 1 ± 0.5 mg. Detailed procedure of carbon and nitrogen stable isotopes ratios quantification is available in Morissette et al. (2019). Results are expressed as part per thousand (‰) noted as $\delta^{13}C$ and $\delta^{15}N$.

Data and statistical analyses

Body length of each individual at each age was back-calculated using the Body Proportional Hypothesis (BPH) with the following formula:

$$L_i = \left[\frac{c+dS_i}{c+dS_c}\right] \times L_c \qquad (1)$$

with L_i and S_i being respectively body length and otolith radius length at age i, L_c and S_c being the same measures at the time of capture, and c and d being respectively the intercept and slope of the regression of body size on otolith DR (Francis 1990). We then considered growth as the difference of body length between age i and i + 1. Furthermore, knowing the age of each individual and the sampling year, we were able to determine the year in which each fish hatched. Hatching year, hereafter called "cohort", was used in further analyses.

All analyses were conducted in R v. 3.4.3 (R Core Team 2017). Several explanatory variables were of interest for both morphometrics and growth analyses such as q-value, body condition, lake and cohort (i.e. the year of hatching, obtained from otoliths). Sex could also be a relevant explanatory variable in our analyses, but it was unknown for two of our populations. Furthermore, among our 12 chosen lakes, nine were in the Saint-Maurice wildlife reserve and three in the Portneuf reserve, including the two lakes for which sex was unknown. To control for a possible influence of the wildlife reserve or sex on our results, we thus performed our analyses on morphology and growth three times, first with all our 12 populations without sex in models, secondly with only nine populations, all belonging to the Saint-Maurice reserve without sex included in our models, and finally with the nine populations of Saint-Maurice with sex included in models. We also made sure that there was no multicollinearity for all of our models by checking the variance inflation factor (VIF < 3, Graham 2003). We controlled residuals before and after each model selection and removed outliers (n = 1 for length analysis at 3 years) when necessary.

Morphometrics analyses

All morphometric analyses were performed using the package geomorph v.3.0.6 (Adams and Otárola-Castillo 2013). To determine which variables affected Procrustes coordinates (i.e. shape), we first performed Procrustes ANOVA (Klingenberg and McIntyre 1998) using the type III ANOVA to compare nested models in order to determine the significance of each variable with a randomized residual permutation procedure (10 000 iterations). The full model comprised the q-value, Fulton index, total body length, the identity of the population and cohort of each individual, as well as interactions between q-value and lake, q-value and cohort and lake and cohort (as along with sex when only considering populations for which this variable was known) as explanatory variables. All variable removals were tested one by one with backward stepwise selection and likelihood ratio tests.

To further characterize shape variation and its determinants, we performed Principal Component Analysis (PCA) on Procrustes coordinates (an analysis also called relative warp analysis when there is no weighting as it is the case here). We only kept the principal components axis (PCs, also called relative warps) that explained at least 5% of the variation in our further analyses as suggested in Zelditch et al. (2004). We then used each of these PCs as response variables in linear models. Full models included all the explanatory variables that remained significant after the stepwise selection performed on the Procrustes ANOVA since they explained shape variation. We then performed backward stepwise selection to identify the significant variables for each model.

Growth analyses

We analyzed growth data using two types of response variables. First, we used size-at-age for each individual from 1 to 5 years old; secondly, we used growth (i.e. the difference of size between two years) from 1 to 5 years old. For both of these types of response variables, we made linear models with q-value, Fulton index, score of otolith reading confidence, the identity of the population and cohort of each individual, as well as interactions between q-value and lake, q-value and cohort and lake and cohort as explanatory variables. We then applied a backward stepwise model selection.

Stable isotopes analyses

To determine how the genetic status influences feeding habits, we split individuals into three categories in accordance with their q-values. Several thresholds were possible to differentiate domestic, wild and hybrid individuals. We considered that individuals with a q-value of 0.2 or lower were wild, 0.8 or higher were domestic and values between 0.2 and 0.8 represented hybrids. We also conducted all of these analyses using a threshold of 0.1-0.9 to ensure that our results did not depend on the chosen threshold (Vähä and Primmer 2006).

For each lake, we ran ANOVAs using $\delta^{13}C$ and $\delta^{15}N$ as response variables and the genetic status assigned as explanatory variable. We then performed Tukey post-hoc tests to characterize more precisely how isotopes ratios differed between groups. To account for multi-testing, we applied a False Discovery Rate (FDR, Pike 2011) correction on these results.

Results

For both morphometrics and growth analyses, results were very similar when excluding lakes from the Portneuf reserve and/or when adding sex in our models. Thus, only the results obtained with all of the 12 lakes are presented (but see Supplementary material Appendix S2.B for the detailed results of the analyses on the subsample of nine Saint-Maurice lakes with and without sex).

Morphometrics analyses

All 18 landmarks were highly repeatable (lowest repeatability score was r = 0.98, 95% CI = [0.97-0.99], $P < 0.001$) and measurement error quantified by Procrustes ANOVA was negligible (SS = 0.005, $P = 0.27$).

All response variables of the Procrustes ANOVA remained significant (all $P \leq 0.04$, Table A1.3) and were kept for the relative warp analyses. Only the first four PCs explained at least 5% of total variance of the PCA (Table 2.1). Explanatory variables that remained significant after model selection are not the same in the four models with PC1 = 36.0%, PC2= 15.6%, PC3 = 7.6% and PC4 = 7.2%. Response variables that remained significant after model selection were not the same in the four models (Table 2.1). The only variable present in all final models was lake, and q-value remained in two final models because of its interactions with lake and/or cohort.

We then determined which morphological characteristics are summarized by each of the four analyzed PCs. PC1 represented mostly body curvature and head orientation (Fig. S2.1A, Fig S2.A4); PC2, 3 and 4 were more reflective of body depth (Fig. S2.1B, C, D).

Growth analyses

For growth analyses, variables remaining significant were different depending on age, and only lake was present in all final models (Table 2.2). Genetic origin (i.e. q-value) significantly influenced growth for three of our five models, in two cases because of its interaction with lake. Size at each age analyses showed slightly different results as lake, cohort and q-value remained significant in final models for all ages, with different combinations of their interactions being also significant (Table 2.2).

Table 2.1 F-values from backward stepwise selection of linear models on morphometric data (relative warp analysis, n = 457).

	Lake:q-value	Cohort:q-value	Lake:Cohort	Lake	Cohort	q-value	Fulton index	Total length	Adjusted R^2
PC1	1.14	0.96	0.83	**3.93**	0.85	0.63	2.61	2.05	*6.6%*
PC2	**2.65**	1.25	**1.97**	**inter**	**inter**	**inter**	**45.86 (-0.004)**	**5.40 (-0.0005)**	*58.8%*
PC3	1.35	0.82	1.33	**6.97**	0.39	0.79	**70.77 (0.004)**	**4.478 (0.0002)**	*38.9%*
PC4	**1.95**	1.15	1.16	**inter**	1.85	**inter**	1.77	0.02	*21.7%*

Significant variables ($p < 0.05$) are in bold. Estimates are provided for significant continuous variables that are not in an interaction. Removal of variables that are in an interaction was not tested, thus we provide no value in these cases and indicate them with the term "inter".

Table 2.2 F-values from backward stepwise selection of linear models on growth (cm/year) and total length (cm) at each age ("YO" = years old).

	n	Lake:q-value	Cohort:q-value	Lake:Cohort	Lake	Cohort	q-value	Fulton index	Otolith reading confidence	Adjusted R^2
Length 1 YO	486	**2.02**	0.73	**2.22**	**inter**	**inter**	**inter**	2.94	0.35	*30.4%*
Length 2 YO	464	1.48	1.31	**1.95**	**inter**	**inter**	**9.58 (1.09)**	1.16	1.89	*24.0%*
Length 3 YO	324	1.13	1.13	**1.77**	**inter**	**inter**	**16.54 (1.93)**	0.36	3.18	*20.5%*
Length 4 YO	197	**2.71**	**2.36**	1.60	**inter**	**inter**	**inter**	0.19	0.00	*24.4%*
Length 5 YO	92	1.16	**3.81**	0.61	**6.94**	**inter**	**inter**	**3.00 (-0.77)**	0.00	*46.8%*
Growth 0-1 YO	486	**2.02**	0.73	**2.22**	**inter**	**inter**	**inter**	2.94	0.35	*30.4%*
Growth 1-2 YO	464	0.37	1.38	**2.23**	**inter**	**inter**	**4.12 (0.50)**	0.23	**5.97 (0.22)**	*17.2%*
Growth 2-3 YO	325	1.12	1.00	1.41	**2.02**	0.36	2.65	0.18	**3.88 (0.17)**	*4.2%*
Growth 3-4 YO	197	**1.96**	0.40	1.41	**inter**	**2.41**	**inter**	0.51	0.35	*16.0%*
Growth 4-5 YO	92	0.67	0.89	0.89	**2.82**	2.17	0.05	0.00	0.48	*16.6%*

Significant variables ($p < 0.05$) are in bold. Estimates are provided for significant continuous variables that are not in an interaction. Removal of variables that are in an interaction was not tested, thus we provide no value in these cases and indicate them with the term "inter".

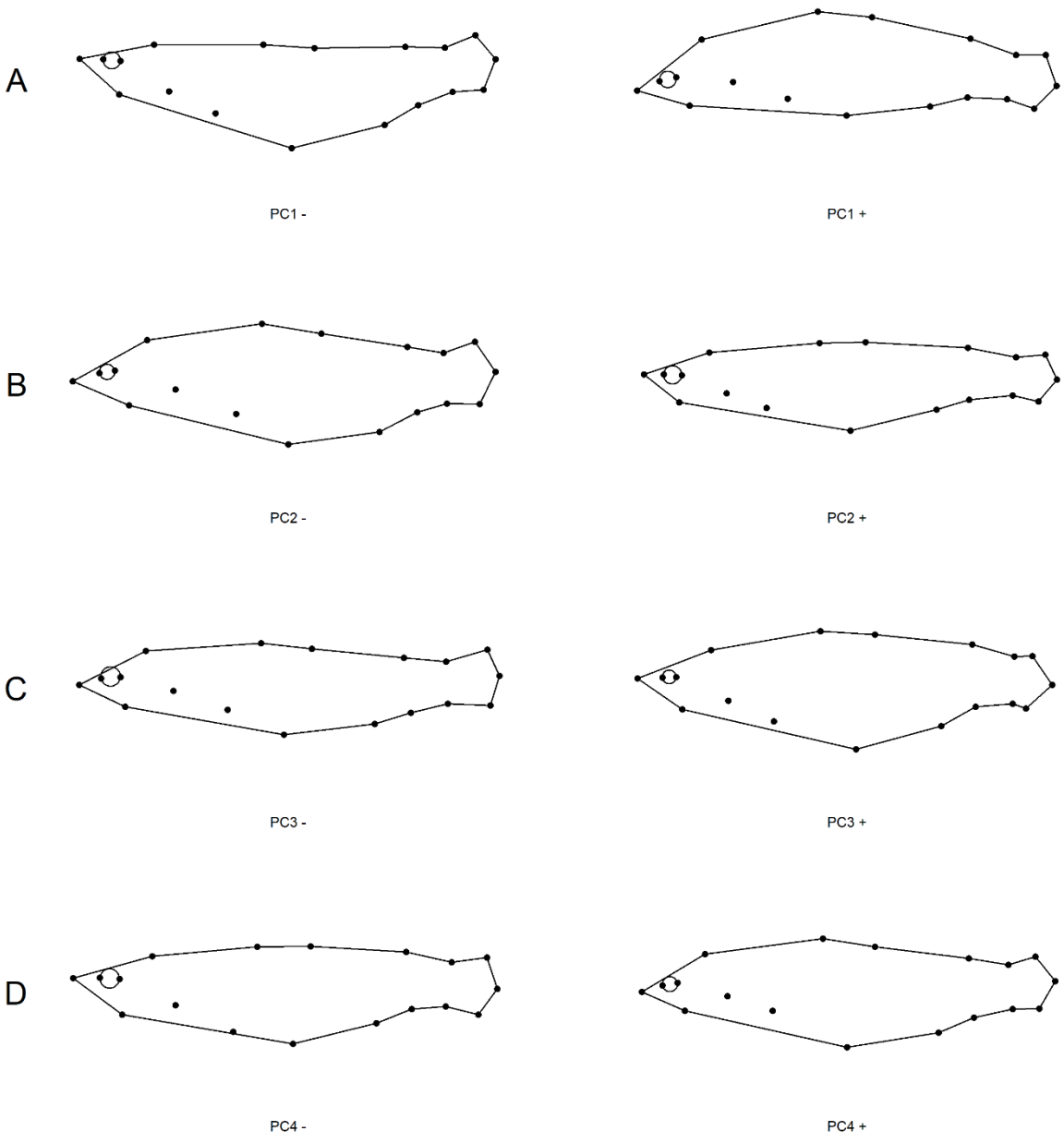

Figure 2.1 Extreme shapes of each PC from PC1 to PC4.

They respectively explain A) 36.0%, B)15.6%, C) 7.6% and D) 7.2% of total shape variation. PC1 mostly reflects body curvature and PC2 to PC4 mostly characterize body depth.

In cases where q-value term was significant in a given model without being in an interaction (2-year-old, 3-year-old length and growth between 1 and 2 years old), its effect was positive, meaning that fish with more domestic genetic background were larger (Fig. S2.A5) and had a higher growth. When it interacted with either lake (size at 1 and 4 years old, growth from 1 to 2 and 3 to 4 years old) or cohort (size at 3, 4 and 5 years old), the direction of effect was variable (Fig. 2.2, Fig. S2.A6).

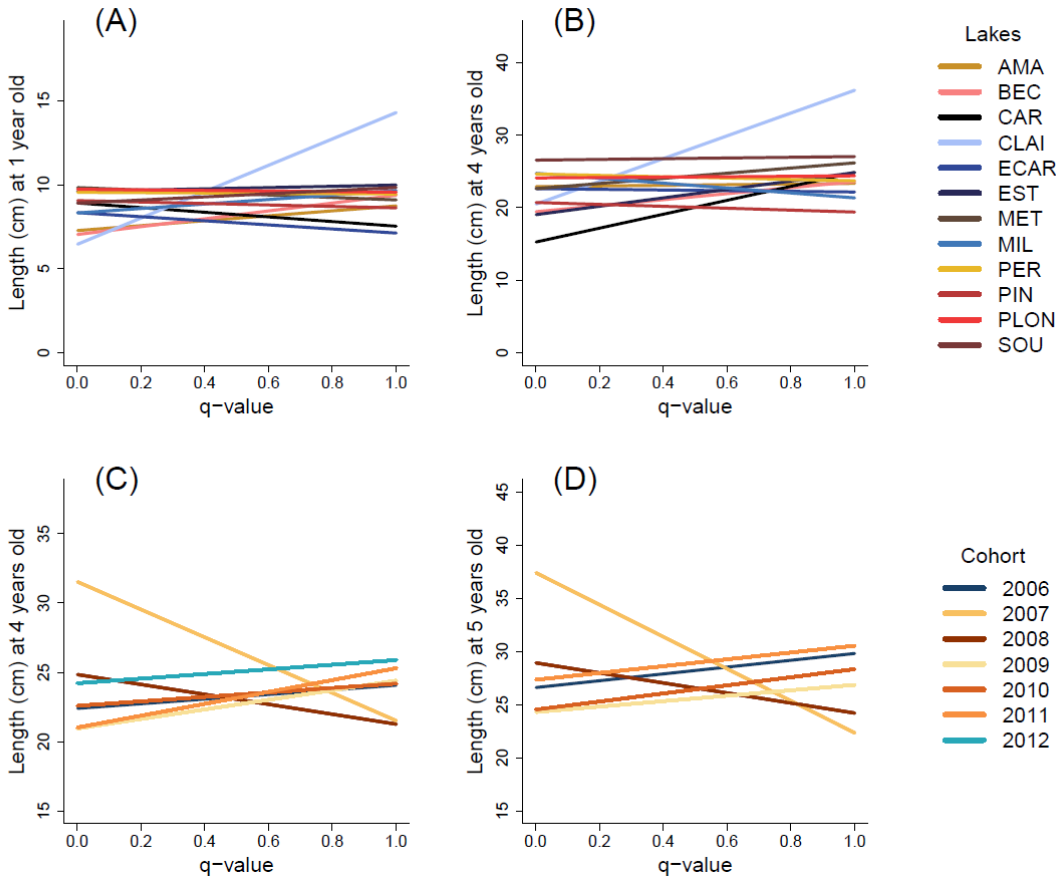

Figure 2.2 Graphs of significant interactions between q-value and lake at (A) 1 year old and (B) 4 years old, and cohort (i.e. hatching year) at (C) 4 years old and (D) 5 years old on total length.

Complete names of lakes can be found in Table S2.A1.

Stable isotopes analyses

Results were the same with the two types of thresholds used to discriminate between domestic, wild and hybrid individuals and we thus only present here results obtained with the 0.2-0.8 threshold (see Table S2.A4 for the 0.1-0.9 threshold).

Genetic origin significantly influenced $\delta^{13}C$ in all lakes and also $\delta^{15}N$ in three lakes out of four (all $P < 0.001$ except for $\delta^{15}N$ in lake MER where $P = 0.18$). Tukey tests showed that there was no difference between wild and hybrid individuals for both $\delta^{13}C$ and $\delta^{15}N$ (Table 2.3, Fig. 2.3, 2.4). However, domestic fish had significantly higher $\delta^{13}C$ than wild individuals in all lakes and also higher $\delta^{15}N$ in three out of four populations (Table 2.3, Figure 2.3, 2.4). Domestic fish also displayed higher $\delta^{13}C$ and $\delta^{15}N$ than hybrids in two out of four populations (Table 2.3, Fig. 2.3, 2.4).

Table 2.3 Results of the Tukey post-hoc tests of the effects of genetic origin on $\delta^{13}C$ and $\delta^{15}N$ ratios.

Lake	Genetic status	$\delta^{13}C$			$\delta^{15}N$		
		lwr	upr	p	lwr	upr	p
AMA	H-D	**-7.68**	**-3.12**	**<0.001**	**-1.01**	**-0.26**	**<0.001**
AMA	W-D	**-7.95**	**-4.84**	**<0.001**	**-1.07**	**-0.56**	**<0.001**
AMA	W-H	-3.14	1.15	0.67	-0.53	0.18	0.69
BEL	H-D	-14.61	1.48	0.20	-2.16	0.85	0.73
BEL	W-D	**-8.07**	**-2.04**	**<0.001**	**-1.58**	**-0.45**	**<0.001**
BEL	W-H	-6.55	9.57	0.97	-1.86	1.15	0.92
MER	H-D	-3.19	-0.20	**0.03**	-1.28	0.19	0.33
MER	W-D	**-2.51**	**-0.71**	**0.00**	-0.47	0.42	0.99
MER	W-H	-1.34	1.50	0.99	-0.18	1.22	0.33
MET	H-D	**-8.44**	**-4.60**	**0.00**	**-1.92**	**-0.33**	**<0.001**
MET	W-D	**-8.63**	**-5.84**	**0.00**	**-1.90**	**-0.75**	**<0.001**
MET	W-H	-2.36	0.93	0.67	-0.88	0.48	0.91

Genetic status (D = domestic; H = hybrid; W = wild) were determined with the 0.2-0.8 threshold of q-values ($q < 0.2$ = W; $0.2 < q < 0.8$ = H; $0.8 < q$ = D). Intervals are based on the Studentized range statistic with a 95% confidence level and are reported in columns "lwr" for the lower interval and "upr" for the upper interval. Values of p are presented here after the application of the False Discovery Rate (FDR) correction. Significant differences between groups ($p < 0.05$, intervals do not overlap 0) are in bold. Names of the lakes : AMA = Amanites; BEL = Belles de Jour; MER = Mercure; MET = Methot.

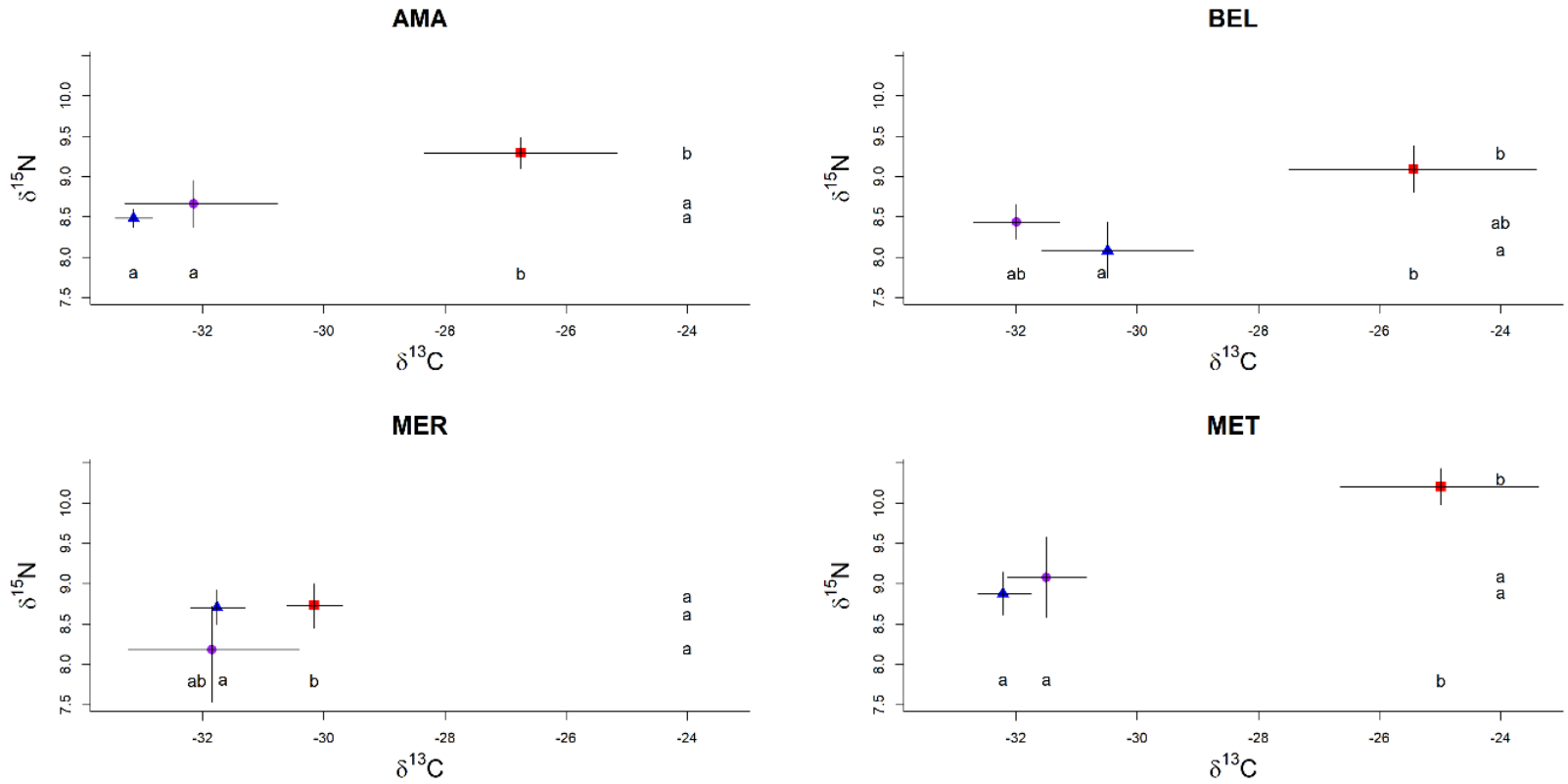

Figure 2.3 **Stable isotopes ratios of $\delta^{13}C$ and $\delta^{15}N$ for Brook Trout in four lakes.**

Red squares represent domestic trout, purple circles hybrids and blue triangles wild trout. Genetic status were determined with the 0.2-0.8 threshold of q-values ($q < 0.2$ = W; $0.2 < q < 0.8$ = H; $0.8 < q$ = D). Letters "a", "b" and "ab" reflect significant differences for $\delta^{13}C$ clusters on the horizontal axis and $\delta^{15}N$ clusters on the vertical axis. Error bars represent 95% confidence intervals. Names of the lakes: AMA = Amanites; BEL = Belles de Jour; MER = Mercure; MET = Methot

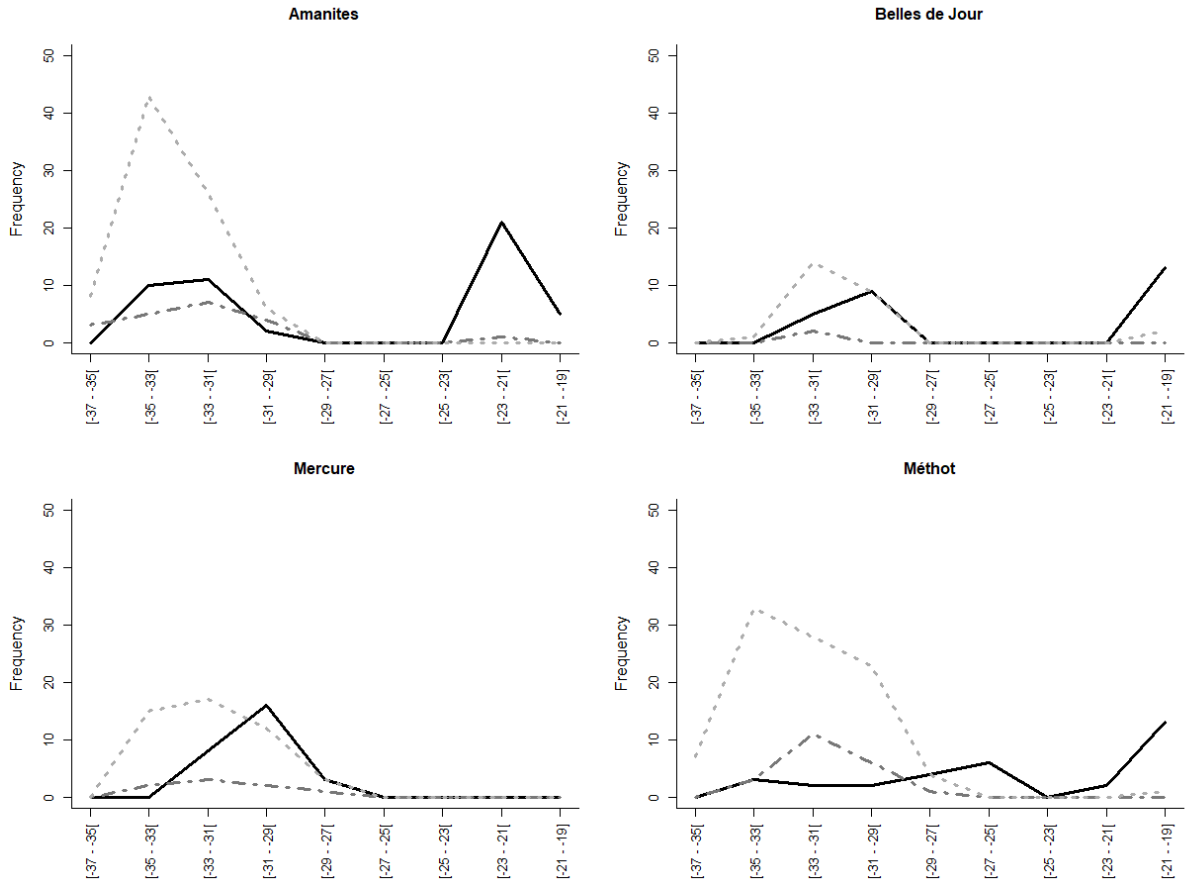

Figure 2.4 **Frequency of individuals of each genetic status along the δ13C gradient of each lake.**

Solid black lines, dot-dash dark grey lines and dashed light grey lines represent respectively domestic, hybrid and wild individuals.

Discussion

The main goal of this study was to assess the effect of genetic origin of Brook Trout on phenotypic traits divergence in stocked populations. A second goal was to assess whether genetic origin influences trophic niche and/or level of individuals. We first found an influence of genetic origin on morphology, growth and size-at-age which varied with environmental conditions. We also established that domestic fish differed from their wild and hybrid counterparts in terms of trophic niche and level, as their isotopic signatures indicated that they were feeding more often on higher trophic level preys found in littoral environments.

Morphology

The main driver of morphological variation was the lake identity, suggesting that shape of individuals was primarily determined by the characteristics of the population they belonged to. This could be partly explained by local environmental differences between lakes. Indeed, lakes within this study system vary in terms of abiotic (e.g. temperature, dissolved oxygen levels, depth, lake area, Marie et al. 2012, Létourneau et al. 2018) and biotic conditions (e.g. presence of competitors, parasitic fauna, Gossieaux et al. 2018) that have been shown to influence morphology (Magnan 1988, Bertrand et al. 2008, Baillie et al. 2016, Zastavniouk et al. 2017). Brook Trout were previously shown to display highly variable morphologies among geographically close lakes (Kazyak et al. 2015) and are known to be phenotypically plastic (Peres-Neto and Magnan 2004, Kazyak et al. 2015, Samways et al. 2015, Zastavniouk et al. 2017). However, it should be noted that Brook Trout populations, including in our study system, exhibit strong genetic differentiation among them (Marie et al. 2010, Lamaze et al. 2012). These genetic differences were linked to some phenotypical differences among populations (Bougas et al. 2010, Crespel et al. 2011, 2012, 2013b) and can even result in strain specific genotype-

environment interactions (Crespel et al. 2013a). Therefore, morphological variation observed here could also be, at least partially, attributable to genetic differences among lakes.

Morphological characteristics that were particularly affected by local conditions in our study were body curvature, head orientation and body depth (Fig. 2.1). Variation in head orientation leads to variation in oral gape axis, which is generally linked to the food type selected by fish, with fish that hunt fast prey having a more terminal orientation while fish that forage or feed on the bottom of lakes have a sub-terminal orientation (Diderich 2006). Body depth depends on swimming lifestyle and maneuverability (Diderich 2006). Similar variations in shape patterns have been observed by Zastavniouk et al. (2017) in a study of Brook Trout where shape variation was mostly explained by population identity, which led the authors to conclude that selection acted differently among the various populations, leading to phenotypic divergence.

A portion of the variance in shape was also explained by interactions between lake and genetic origin of fish. This result suggests that genetic background influenced body shape (here mostly body depth, Fig. 2.1B and D) in some environments more than others (e.g. genetic by environment effects, also shown in Harbicht et al. 2014). These morphological differences can be genetically-based, as shown by previous studies (Taylor and McPhail 1985, Swain et al. 1991, Fleming et al. 1994, Fleming and Einum 1997, Pulcini et al. 2013). However, they can also possibly be attributable to the long lasting effect of early-life rearing conditions (hatcheries vs wild) which are strong determinants of body shape (Beacham 1990, Fleming and Einum 1997, von Cramon-Taubadel et al. 2005, Samways et al. 2015). Another possible explanation for the morphological difference between domestic and wild fish is that they reflect ecological differences in terms of niche occupation and/or feeding habits as suggested by our results on stable isotopes (see further discussion below). Previous studies on Brook Trout revealed different morphs in a same population depending on individual feeding habitats (Bourke et al. 1997, Dynes et al. 1999, Bertrand et al. 2008, but see Samways et al. 2015). Domestication has been shown to impact behavioral traits associated to feeding in salmonids (reviewed by

Huntingford 2004) and could thus lead to morphological differentiation if individuals exploit different food sources and niches according to their genetic origin.

Growth

Growth and size-at-age also varied mostly depending on population identity. More specifically, our analyses showed an effect of lake for every age classes, indicating that the environment in which individuals lived was the main determinant of their growth and size. Again, this is likely a consequence of either different environmental conditions among lakes (Yamamoto and Morita 2002), genetic differentiation among lakes (Crespel et al. 2012) or a combination of both with variable genotype-environment interactions (Crespel et al. 2013a). Furthermore, size was also influenced by cohort at each age, suggesting that early environmental conditions may be an important determinant of size at all ages (Jonsson and Jonsson 2014, but see Granier et al. 2011, Lee et al. 2013), and that favorable environments during early life could provide a long-lasting growth advantage to individuals (Petersson et al. 1996).

Growth and size-at-age were also influenced by genetic origin of individuals, which influenced growth for three age classes out of five, and size at every age classes analyzed. Again, genetic effect was mostly dependent on environmental conditions, either spatially (e.g. interaction with lake) or temporally (e.g. interaction with cohort), At 2 years old for growth and 2 and 3 years old for size-at-age, however, the genetic effect was significant independently of environment and indicated that domestic genetic background resulted in more pronounced growth and size at age. This is in line with previous findings (Fleming and Einum 1997, McGinnity et al. 1997, 2003, Tymchuk et al. 2006, Reinbold et al. 2009, Solberg et al. 2013a, 2013b) and likely a result of the artificial selection to produce fast-growing individuals in hatcheries (Petersson et al. 1996, Huntingford 2004). The observation that genetic background affected growth mainly at an early stage may suggest that growth advantage disappeared with age, perhaps due to a lower survival

of domestic fish in the wild (Solberg et al. 2013b), and/or because they are more likely to be harvested during recreational fishing (Härkönen et al. 2014, Uusi-Heikkilä et al. 2017). It is also possible that it becomes harder to detect an effect of genetic origin in older age classes because our smaller sample sizes, and thus statistical power decrease. Still, despite its equivocal effect on growth in older age classes, genetic origin impacted size at each age in our analyses, as domestic fish were larger than wild individuals. This result can be explained by artificial selection leading to genetically-based differences in growth between domestic and wild fish (Fleming and Einum 1997, McGinnity et al. 1997, 2003, Tymchuk et al. 2006, Reinbold et al. 2009, Solberg et al. 2013a). This could also be due to an early boost since hatchery-reared fish were fed *ad libitum* before being released and are thus larger during early life stages (Petersson et al. 1996). Body size have been shown to influence trophic levels in Brook Trout with larger individuals consuming larger prey (Glaz et al. 2012, 2014) and it is thus possible that domestics stay larger than wild fish because of early difference in body size, even though they do not maintain higher growth rates.

Trophic niche and trophic level

Our results showed that domestic trout differed from their wild counterparts both in terms of trophic level and trophic niche. More specifically, $\delta^{13}C$ ratios showed that domestic individuals were feeding consistently in more littoral habitats than wild and hybrid fish. This difference in trophic niche could be due to behavioral differences induced by domestication leading to different preferences in habitat selection (Mittelbach et al. 2014). In the same region as our study system, it has been shown that Brook Trout select preferentially littoral trophic niches and can shift their diet to forage in the pelagic zone when environment is disturbed (Glaz et al. 2014), or in presence of competitors (Magnan 1988, Tremblay and Magnan 1991). This suggests that domestic fish could have displaced wild individuals from littoral niches. Domestication often increases boldness levels (Huntingford 2004, Mittelbach et al. 2014) and it is possible that bolder domestic fish outcompeted wild individuals and took over littoral habitats. This could be

accentuated by the fact that domestic trout are larger at every age, which could give them an advantage over wild fish in intraspecific competition (McGinnity et al. 1997, 2003). A closer look at the feeding niche distribution of each genetic category shows that wild and hybrid individuals almost strictly feed in pelagic niches while domestics feed in both pelagic and littoral environments (Fig. 2.4). This pattern could be explained by an age structure in trophic niche for domestics, with some age classes feeding in littoral zone and other age classes feeding in pelagic environment. However, supplementary analyses showed that size is not related to $\delta^{13}C$ differences within domestic individuals (Table S2.A5, Fig. S2.A7).

In three of our four populations, $\delta^{15}N$ ratios showed that domestic trout displayed higher trophic levels than wild fish. The difference in trophic niche probably explains this pattern since prey tend to be larger in littoral environments (Vander Zanden et al. 2006). Moreover, body size has been shown to positively correlate to $\delta^{15}N$ in Brook Trout (Glaz et al. 2012, 2014). Thus, there is probably a link between our results on growth and on trophic niche and level. Larger size of domestic fish at each age may provide a competitive advantage to take over littoral habitats and feed on larger preys, which in turn allow them to maintain their size advantage.

Interestingly, hybrids clustered either closer to wild trout or had an intermediate position in terms of trophic level or niche. Hybrid fish shared the same niche as wild individuals in two populations and were not different from either wild or domestic fish in two other populations. However, we note that the two populations in which hybrids were not different from wild or domestic individuals had very low numbers of hybrids (Table S2.A2). In these lakes, we thus had mode limited statistical power to analyze this group, which is a possible explanation for the absence of difference between hybrids and other groups. The similarity between hybrids and wild individuals suggest that rearing conditions are more important than genetic origin in shaping feeding habits, since both wild and hybrid fish, unlike domestic trout, were reared in the same environment. An alternative explanation is that genetic differences between groups influence their trophic habitat use, but that niche occupation behavior is governed by genes that

have a dominance-recessive pattern, with wild genes being dominant. For instance, mechanisms of dominance were shown to affect traits such as transcription regulation in a context of hybridization in Brook Trout (Bougas et al. 2010). Hybrid traits are difficult to predict in natural systems (Granier et al. 2011) and their heritability can vary according to environmental conditions (Crespel et al. 2013a). It is thus possible that in other contexts or populations, hybrids would cluster differently than what we observed here.

Conclusion

Overall, our results showed an effect of genetic origin of individuals on phenotypes and feeding habits, which varied depending on population-specific attributes, both at the scale of the lake and at the scale of the cohort. Domestic trout seem to grow larger and faster than wild fish and to monopolize the best quality feeding niches. In other salmonids, stocked domestic fish have been showed to displace wild populations (Morissette et al. 2019), possibly because of their size advantage (McGinnity et al. 1997, 2003). This size and growth advantage of domestic fish appears in early-life stages in our results and likely leads to domestics outcompeting wild individuals. Thus, limiting stocking to fish that are already large enough to be caught by anglers (e.g. using put-and take rather than put-and-grow stocking practices) could reduce competition in early-life stages for wild fish. In addition, assuming that Brook Trout spend most of their time in their feeding habitat, we can speculate that focusing angling pressure on littoral habitats could help alleviate fishing pressure on wild individuals and could thus limit the impacts of stocked fish by controlling their population. Accounting for risks of wild population displacement from their preferential niches when stocking is an important step for an effective management Brook Trout and salmonids in general. Finally, since environment seems to strongly influence phenotype, but also the relationship between genetic background and phenotype, further research about environmental conditions would be needed to better identify the conditions in which such phenotypic divergence should be enhanced or inhibited.

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CHAPITRE 3
RELATIONS HÔTES-PARASITES

Description de l'article et contribution

Malgré l'importance écologique des relations hôtes-parasites dans les populations naturelles, les conséquences des ensemencements sur la faune parasitaire n'ont été que très peu étudiées. Pourtant, des travaux sur les impacts des échappées de saumons domestiques sur l'occurrence de certains types de parasites dans les populations naturelles, ainsi que les effets potentiels d'un relâchement de la pression de sélection en pisciculture, suggèrent qu'ensemencer des poissons issus de l'élevage dans des populations naturelles pourrait y affecter les relations-hôtes parasites. L'objectif de cet article était donc de déterminer comment l'introduction de poissons domestiques pouvait modifier les relations hôtes-parasites, dans un premier temps au niveau individuel, puis au niveau populationnel. Les résultats ne montrent aucun effet de l'origine génétique des individus sur le parasitisme. Cependant, les populations les plus introgressées présentent des niveaux de prévalence et de diversité de parasites plus faibles. L'absence d'effet au niveau individuel semble suggérer que les effets observés au niveau populationnel ne sont pas dus directement à des conséquences de la domestication, mais plutôt à des variables confondantes non mesurées, très probablement environnementales.

Pour cet article, j'ai participé à la collecte des données (2015-2016) et j'ai effectué le travail de génotypage. Je tiens à remercier Raphaëlle Dubois et Nicolas Bousquet pour leur aide concernant le travail de terrain. Je remercie aussi Raphaëlle Dubois et Xavier Dallaire qui ont effectué tout le travail de dissection afin de dénombrer et identifier les parasites internes. L'élaboration des idées s'est faite en collaboration avec Dany Garant. J'ai analysé les données et rédigé le manuscrit. Dany Garant a guidé une partie des analyses et révisé plusieurs versions du manuscrit. Louis Bernatchez et Pascal Sirois ont révisé le manuscrit.

Introgressive hybridization between wild and domestic individuals and its relationship with parasitism in Brook charr *Salvelinus fontinalis*

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Abstract

The effects of introgression on parasitism in Brook charr *Salvelinus fontinalis* were investigated in 28 lakes with various levels of stocking in Québec, Canada. No effect of genetic background on parasitism was found at the individual level. Body length seemed to explain most of the variation observed at this level, with largest fish being more infected. However, lakes with the greater average domestic genetic background were found to display significantly lower parasite prevalence and diversity. Since our results indicate no effect of domestic genes at the individual level, the negative association with introgression found at the population level may be mainly attributed to differences in intrinsic environmental quality of lakes (*e.g.* fishing pressure, availability of food resources, abiotic characteristics).

Keywords: Admixture; Hosts-parasites relationship, Hybridization; Salmonids; Stocking; Trout

Introduction

Human activities are nowadays a major threat to natural populations worldwide and are recognized as one of the main causes of decline of many species (Sanderson *et al.*, 2002; Halpern *et al.*, 2008; Goudie, 2013). These declines can occur because of multiple factors related to human activities including habitat loss, introduction of invasive species, pollution or overexploitation (Wilcove *et al.*, 1998). As a result, conservation actions are widely applied to counteract these negative effects (Sanderson *et al.*, 2002). For instance, stocking is a practice widely used to prevent collapses of exploited fish populations (Laikre *et al.*, 2010; Soorae, 2013). It often relies on using hatchery-reared fish to supplement wild populations. However, these farmed fish are affected by domestication, which is defined by Price (1999) as "the process by which a population of animals becomes adapted to man and to the captive environment by genetic changes occurring over generations". Domestication is caused by artificial selection, deliberate or not (Perry *et al.*, 2005; Uusi-Heikkilä *et al.*, 2017), which is due the selective pressures encountered in artificial environments (*e.g.* hatcheries) and can happen very quickly, sometimes after only one or two generations of captivity (Christie et al., 2012; Fraser et al., 2018).

Stocking with farmed fish often leads to hybridization and genetic introgression of exogenous alleles in wild populations (Rhymer & Simberloff, 1996; Laikre *et al.*, 2010). While introgressive hybridization can sometimes increase genetic diversity (Marie *et al.*, 2010), it is generally perceived as a threat to natural populations. For instance, introgression can reduce the fitness of hybrids (Araki *et al.*, 2007, 2009), cause the loss of local adaptations (Allendorf *et al.*, 2001; Laikre *et al.*, 2010), and ultimately compromise the viability of wild populations (Rhymer & Simberloff, 1996; McGinnity *et al.*, 2003; Araki *et al.*, 2009; Muhlfeld *et al.*, 2014). Thus, the cost/benefit of stocking is widely debated in the literature because despite its conservation purpose, it can ultimately impede the recovery of supplemented populations (Rhymer & Simberloff, 1996; Brown & Day, 2002; Laikre *et al.*, 2010).

A fitness component of fish that should be greatly affected by stocking is immunity. First, parasitism and diseases cause high mortality in hatcheries and thus medication is extensively used to limit the presence and impact of pathogens (Scholz, 1999; Duston & Cusack, 2002). Such intense use of medication can lead to a relaxed selection on pathogens resistance and greater susceptibility to parasitic infection (Bakke & Harris, 1998; van Oosterhout *et al.*, 2007; Naish *et al.*, 2008; Lamaze *et al.*, 2014). Domestic and introgressed fish can thus be more vulnerable to diseases and parasites than wild individuals (van Oosterhout *et al.*, 2007; Consuegra & de Leaniz, 2008; Naish *et al.*, 2008). Also, domestic fish grow larger and faster than wild fish, since growth is a trait under strong selection in hatcheries (Thorpe, 2004; Solberg *et al.*, 2013) and could thus have poorer immunity because of a trade-off among these components (Lamaze *et al.*, 2014; see also Mangel & Stamps, 2001).

At the population-level, stocking should also impact hosts-parasites relationships in different ways. Domestic individuals brought in the wild can become vectors for the introduction of new parasites (Wootten, 1973; Naish *et al.*, 2008; but see Valtonen & Koskivaara, 1994) and/or create favourable conditions for their establishment (Krkošek *et al.*, 2006; Krkošek, 2017). Additionally, since supplementing a lake implies increased density of fish (*i.e.* potential hosts), the transmission of parasites can be facilitated and prevalence of infection (*i.e.* the proportion of infected hosts in a population) could increase (van Oosterhout *et al.*, 2007). Yet, despite the importance of parasitism in the dynamics and viability of populations, the impacts of stocking on parasite communities have rarely been monitored in supplemented populations and the relationship between parasitism and genetic introgression has received very little attention in the literature. Previous studies conducted at the interspecific level showed equivocal results, with hybrid fish displaying either a poorer (Dupont & Crivelli, 1988), intermediate (Le Brun *et al.*, 1992; Bakke *et al.*, 1999; Kalbe & Kurtz, 2006) or better (Šimková *et al.*, 2012, 2013; Krasnovyd *et al.*, 2017) resistance to parasites than the parental strains. At the intraspecific level, some studies aimed at understanding how hybridization between host strains belonging to different geographic areas shapes parasitism (*e.g.* Kalbe & Kurtz, 2006; Kalbe *et al.*, 2016) and others showed that domestication could negatively affect the parasite resistance of farmed fish

(*e.g.* van Oosterhout *et al.*, 2007; Consuegra & de Leaniz, 2008), yet only a few investigated the effects of genetic introgression of domestic genes on parasitism. For instance, Currens *et al.* (1997) showed that introgression of exogenous genes through farmed fish stocking decreased the individual resistance to a myxosporean parasite in a population of rainbow trout (*Oncorhynchus mykiss* (Walbaum 1792)). Also, Lamaze *et al.* (2014) suggested that, after stocking with farmed fish, individuals with a more domestic background were more heavily infected by parasites in Brook charr *Salvelinus fontinalis* (Mitchill 1814). These results suggest that introgression could lower the individual resistance to parasites after stocking.

The main objective of this study was to investigate how stocking and genetic introgression affected the hosts-parasites relationships in *S. fontinalis* from 28 lakes located in Québec, Canada. *Salvelinus fontinalis* is the most important species for recreational angling in Québec and several lakes are heavily stocked each year (see Marie *et al.*, 2010 for details). Lakes with variable stocking intensity and introgression levels were selected and different parameters related to parasitism were evaluated at the individual and population level. More specifically, at the individual level, the relationship between introgression and infection status (*i.e.* being infected or not) and intensity of the infection (*i.e.* number of parasites carried by infected individuals) was investigated. At the population level, the variation of diversity of parasite communities and prevalence among lakes were analysed as a function of introgression level and environmental variables.

Material and methods

Sampling sites and procedures

Sampling has been conducted in three wildlife reserves (Portneuf, Mastigouche and Saint-Maurice) in Québec, Canada (Fig. 3.1) in 2015 (all reserves) and 2016 (Saint-Maurice only). *Salvelinus fontinalis* were sampled in 28 lakes (4 in Portneuf, 6 in Mastigouche and 18 in Saint-Maurice) using experimental gill nets in May, June and July 2015, and in June and July 2016. Lakes that were sampled had a known history of stocking since 1964 (provided by the Ministère des Forêts, de la Faune et des Parcs). Some of these lakes were stocked intensively for decades while others were not stocked for years (Table 3.1). The number of sampled fish per lake varied between 32 and 67 (Table 3.1) and, overall, 731 fish were sampled in 2015 and 509 in 2016. Fish were euthanized with clove oil right after their capture. Each fish was weighed (± 1 gram), measured (total length ± 1 mm) and sexed by observation of the gonads during dissection in the field. Individuals for which sex could not be determined were noted as "indeterminate" since they were in most cases very small and had not reached the stage of gonad development. Digestive tracts were preserved in formaldehyde 4% until being dissected in the laboratory for parasites analyses. Adipose fin of each fish was preserved in 95% ethanol until DNA extraction and genotyping. All protocols and procedures employed were reviewed and approved by the Ministère des Forêts, de la Faune et des Parcs.

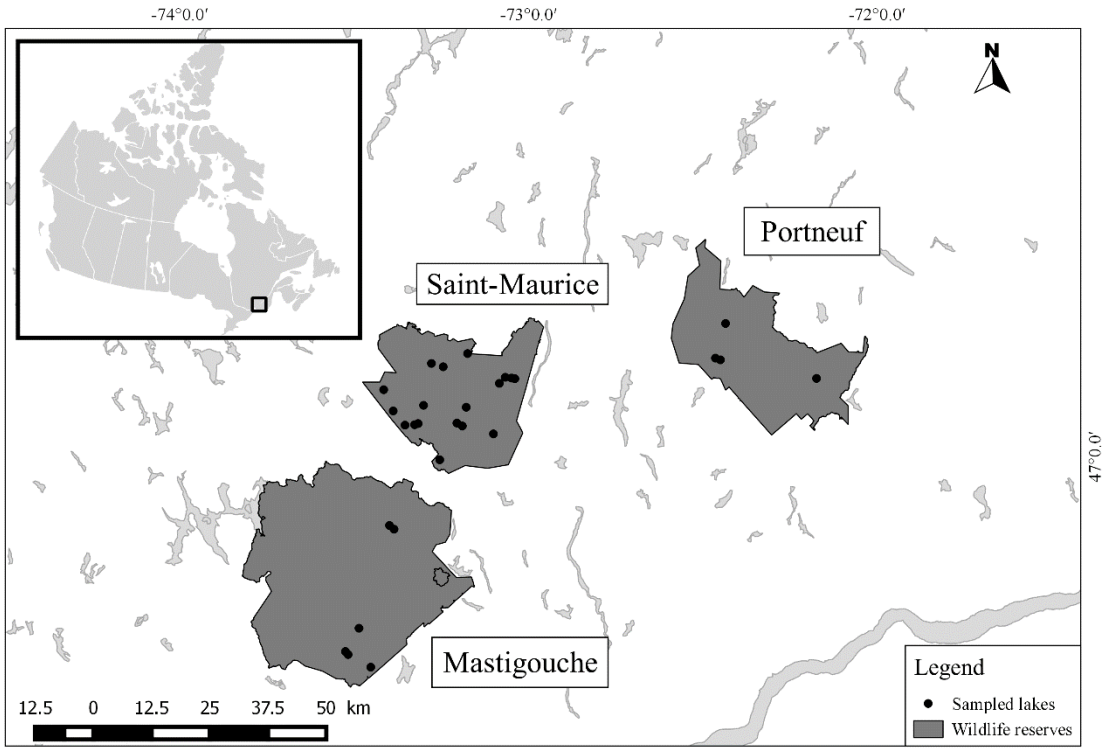

Figure 3.1 Location of sampled lakes in the wildlife reserves of Portneuf, Mastigouche and Saint-Maurice in Québec, Canada.

Parasitism analyses

In the field, each fish was inspected for the presence of external parasites when captured. In the laboratory, the digestive tract of each fish was dissected to investigate the presence of internal parasites. Parasites were identified to the genus level (Northcote, 1957). All parasites (internal and external) were pooled together for all analyses (analyses conducted on separate groups provided similar results – see Supporting information, Tables S3.A1 and S3.A2). Prevalence was estimated for each lake and intensity of infection for each parasitized individual, according to the definitions of Bush *et al.* (1997). Infection status was defined as a binary (0 or 1) variable indicating whether a fish was infected or not by at least one parasite.

The intensity of infection was determined by counting all parasites observed for each fish. When too many parasites of one genus were present to be counted accurately, a fixed value of 500 for external parasites and 300 for internal parasites was attributed. These values were chosen to be slightly greater than the maximum number of parasites actually counted in each category (maximum count of 432 for external parasites, 220 for internal parasites). Due to important overdispersion in the distribution of the intensity of infection variable, it was treated as a categorical variable rather than as a continuous one. To do so, infected fish were divided in two categories according to the median number of parasites per fish (the median is 14.5 so the limit was set to 15 parasites). The heavily infected group thus included fish that carried 15 parasites or more, whereas the lightly infected group comprised fish that carried less than 15 parasites.

Stocked fish in the sampled lakes came from different hatcheries and consisted either of strains kept in captivity for multiple generations (*e.g.* Jacques Cartier hatchery) in the Portneuf reserve or of hybrid strains (*e.g.* Lac des Écorces and Saint-Alexis des Monts hatcheries: crosses using domestic fish and wild fish collected each year from Bourassa lake, which is located in Mastigouche reserve) in the Mastigouche and Saint-Maurice reserves. Samples from each hatchery were thus collected (Jacques Cartier "JC", n = 53, Saint-Alexis des Monts "A", n = 80, Lac des Écorces "E", n = 40) and from lake Bourassa ("B", n = 40).

Table 3.1 Summary of information for sampled lakes in this study.

Y_S	Reserve	Lake	n	Y_{FS}	Y_{LS}	Y_{SS}	N_{SE}	N_{ST}	Area	N_{ST}/ha	q-value ± s.d.	Strain
2015	MAS	Demarest (DEM)	66	1999	1999	16	1	1000	5.2	192.31	0.01 ± 0.02	E+B+A
2015	MAS	Head (HEAD)	46	1972	1972	43	1	2500	9.7	257.73	0.05 ± 0.07	E+B+A
2015	MAS	Cerné (CER)	67	NS	NS	NS	0	0	13.2	0	0.06 ± 0.09	E+B+A
2015	MAS	Chamberlain (CHAMB)	41	1972	2006	9	9	17250	18.4	937.50	0.16 ± 0.27	E+B+A
2015	MAS	Deux Étapes (DETP)	40	1972	2012	3	22	44861	12.3	3647.24	0.28 ± 0.4	E+B+A
2015	MAS	Pitou (PIT)	40	1971	2013	2	23	20971	8	2621.38	1 ± 0	E+B+A
2015	PN	Sorbier (SOR)	46	NS	NS	NS	0	0	5	0	0.01 ± 0.02	JC
2015	PN	Langoumois (LANG)	41	NS	NS	NS	0	0	10	0	0.01 ± 0.02	JC
2015	PN	Main de Fer (MDF)	38	NS	NS	NS	0	0	16	0	0.02 ± 0.13	JC
2015	PN	Caribou (CAR)	32	2008	2013	2	11	2850	5	570.00	0.2 ± 0.26	JC
2015	STM	Corbeil (CORB)	43	1969	1971	44	2	5000	9.5	526.32	0.02 ± 0.04	E+B+A
2015	STM	Brown (BRO)	63	1966	1975	40	4	31250	273	114.47	0.03 ± 0.04	E+B+A
2016	STM	Courbé (COUR)	40	1968	2015	1	22	218965	105.1	2083.40	0.06 ± 0.09	E+B+A
2015	STM	Portage (PORT)	40	1979	1990	25	11	48964	46.9	1044.01	0.07 ± 0.09	E+B+A
2016	STM	Clairval (CLAI)	40	1973	2015	1	21	61595	24.9	2473.69	0.15 ± 0.26	E+B+A
2015	STM	Milord (MIL)	44	1969	2005	10	16	54850	46.7	1174.52	0.16 ± 0.27	E+B+A

Y_S	Reserve	Lake	n	Y_{FS}	Y_{LS}	Y_{SS}	N_{SE}	N_{ST}	Area	N_{ST}/ha	q-value ± s.d.	Strain
2016	STM	Epervier (EPER)	40	1998	2015	1	9	14900	12.8	1164.06	0.18 ± 0.28	E+B+A
2016	STM	Plongeon-Huard (PLON)	40	1983	2015	1	16	14932	5.3	2817.36	0.22 ± 0.23	E+B+A
2016	STM	Ecarté (ECAR)	41	1979	2014	2	18	27459	6.3	4358.57	0.22 ± 0.28	E+B+A
2015	STM	Soucis (SOU)	41	1976	1976	39	1	750000	267.5	2803.74	0.25 ± 0.34	E+B+A
2016	STM	Est (EST)	40	1980	2015	1	14	27398	12.2	2245.74	0.46 ± 0.47	E+B+A
2015	STM	Perdu (PER)	43	1964	1990	25	16	50668	22.1	2292.67	0.49 ± 0.34	E+B+A
2016	STM	Bec-Scie (BEC)	41	1983	2015	1	20	28850	9.7	2974.23	0.66 ± 0.43	E+B+A
2016	STM	Sud (SUD)	40	1979	2015	1	18	18868	6.4	2948.13	0.77 ± 0.39	E+B+A
2016	STM	Pin (PIN)	64	1991	2015	1	13	14897	5.9	2524.92	0.81 ± 0.36	E+B+A
2016	STM	Boucher (BOUCH)	40	1964	2014	2	34	136049	25.1	5420.28	1 ± 0	E+B+A
2016	STM	Cardinal (CARD)	40	1969	2014	2	24	57726	7.1	8130.42	1 ± 0	E+B+A
2016	STM	Hamel (HAM)	43	1965	2015	1	31	69893	10.7	6532.06	1 ± 0	E+B+A

Note. A: Saint-Alexis des Monts; area: lake area; B: Bourassa lake; JC: Jacques Cartier. NS: lake not stocked; MAS: Mastigouche; n: number of genotyped S. fontinalis; NSE: number of stocking events; NST ha−1: stocking density; NST: number of fish stocked; PN: Portneuf; q: mean q-value of each lake; STM: Saint-Maurice; strain: domestic strains used in analyses: E: Lac des Écorces; Year: Sampling year; YFS: year of first stocking event; YLS: year of last stocking event; YSS: number of years between last stocking event and sampling.

Genetic analyses

DNA was extracted from clips of adipose fins (3 mm²) using a slightly modified version of Aljanabi & Martinez (1997) salting out method. In brief, 44 µl of 20% SDS (1.75% final concentration) and 20µl of proteinase K (790 µg/ml final concentration) were used for tissue digestion and samples were incubated overnight at 60°C. A volume of 300 µl of saline solution (5M) was added and samples were vortexed 1 min. Samples were then centrifuged 30 min at 10 400 rpm. DNA precipitation was performed using 600 µl isopropanol for 30 min. Samples were then centrifuged 20 min at 13 200 rpm at the room temperature. A solution of -20°C ethanol 70% was used to wash the pellets twice with a 10 min centrifugation at 13 200 rpm between these two steps. The pellets were finally diluted in 200 µl of sterile water. The quality and concentration of DNA in the samples were then controlled on 1% agarose gel.

All sampled individuals from lakes and hatcheries were genotyped at 20 microsatellite loci (Supplementary material, Table S3.A3). GeneAmp PCR 9700 and SimpliAmp Thermal Cycler thermocyclers (ThermoFisher Scientific) were used to amplify microsatellites with the following 10 µl reaction mixture: 10 mM Tris-HCl (pH 9.0); 50 mM KCl; 0.1% Triton X-100; 1 or 1.2 or 1.5 mM $MgCl_2$ (see Table S3.A3 for details); 0.2 mM of each dNTPs; 0.4 mg BSA; 0.6 mM fluorescent forward primer; 0.6 mM reverse primer; 0.25 U/µl Taq and 5 ng DNA template. PCR conditions consisted of an initial denaturation step of 6 min at 96°C; then 30-35 cycles of 45 sec at 96°C, an annealing phase of 30 sec at 48°C-62°C (see Table S3.A3 for details) and 45 sec at 72°C; and finally after the last cycle an elongation step of 7 min at 72°C.

Microsatellite loci were analysed using four multiplexes (see Table S3.A3 for details). PCR products of loci from a same multiplex were pooled and 1 µl of this mixture was used for genotyping with 0.15 µl of GeneScan 600 LIZ size standard (Applied Biosystem) and 8.85 µl of Formamide Hi-Di (Applied Biosystem). PCR products were visualized on an AB3130xl

automated DNA sequencer (Applied Biosystem) and alleles lengths were determined using Genemapper v4.1 (Applied Biosystem).

Data were checked for genotyping errors and an error rate was calculated for each locus. Hardy-Weinberg equilibrium and linkage disequilibrium were assessed with a Bonferroni correction using GENEPOP v. 4.3 (Rousset, 2008). The presence of null alleles for each locus, allelic richness and expected and observed heterozygosity were determined using CERVUS v. 3.0.7 (Kalinowski *et al.*, 2007).

The genetic origin and level of introgression (q-value) of each individual was determined using STRUCTURE v. 2.3.4 (Pritchard *et al.*, 2000). The q-values vary between 0 and 1, respectively designating pure wild and pure domestic individuals. Analyses were performed for each lake using the fish sampled and hatchery fish used to stock the lake (Table 3.1). The number of populations (K) was fixed according to the number of populations (defined as: lake + number of hatcheries used for stocking) likely present in a given lake (K = 2 for Portneuf lakes, K = 4 for Mastigouche and Saint-Maurice).

Model variables and statistical analyses

Seven different types of parasites were found (Table S3.A4) in sampled lakes. The number of parasites genera per lake was used as an indicator of parasite diversity. Catch per unit effort has been widely used to estimate fish abundance and has been shown to be a reliable predictor of density with different fishing methods and in different species (Sanders & Morgan, 1976), including *S. fontinalis* (Bergman *et al.*, 2011). Therefore, the catch per unit effort was used as a proxy of the density of fish in the sampled lakes and calculated it as follows:

$$D_{is} = \frac{n_s}{f_i \times t_i} \quad (1)$$

where D_{is} is the proxy of density for lake i for species s, n_s the number of fish of the species s caught, f_i the number of nets used on the lake i and t_i the cumulative fishing time on the lake i.

White suckers *Catostomus commersonii* Lacepède 1803 were also caught as bycatches. This species is a well-known competitor of *S. fontinalis* that can affect hosts-parasites relationships when present (Dubois *et al.*, 1996). The presence of *C. commersonii* was recorded for each lake and their density was estimated using equation (1).

Body length and mass of each individual were used to estimate body condition using the Fulton index (Cone, 1989), which is calculated with the equation: $K = 100 \times \text{weight}/\text{length}^3$. This index correlates with visceral fat and relative liver glycogen in *S. fontinalis* (Crespel *et al.*, 2013) and is thus a reliable indicator of energy storage.

Infection status, intensity of infection and prevalence data were analysed using generalized linear mixed models (GLMM) with a binomial error distribution. The diversity of parasites (*i.e.* number of parasites genera per lake) was analysed using a generalized linear model (GLM) with a Poisson error distribution. All models were simplified using a backward stepwise model selection approach. For individual analyses (*i.e.* infection status and intensity of infection), mass, total length, q-value, sex, and Fulton index were included as fixed effects. An interaction between sex and Fulton index was also included as an additional fixed effect, since body condition can vary differently in males and females (Sutton *et al.*, 2000). Lake and reserve identities were used as random effects to account for hierarchical data structure. For population analyses (*i.e.* prevalence and diversity of parasites), lake area, *S. fontinalis* density, *C. commersonii* density, mean q-value of the lake (mean of the q-values of all *S. fontinalis* in a

given lake), mean total length of *S. fontinalis* and mean Fulton index of *S. fontinalis* were included as fixed effects and reserve identity was included as a random effect. Since the random effect was not significant for the number of parasite species model, it was removed and this variable was analysed with a GLM. All continuous independent variables were standardized (mean = 0, variance = 1). Multicollinearity was accounted for and, as a result, mass was removed from analyses at the individual level because of strong collinearity with length (all VIF < 3; Graham, 2003). All analyses were performed using R version 3.3.2, also using the packages lme4 to fit GLMMs (Bates *et al.*, 2015) and piecewiseSEM to calculate R^2 (Lefcheck, 2016).

Results

The mean genotyping error rate was 0.62% and error rates varied between 0% (for 11 loci) and 2.5%. All loci were polymorphic with allelic richness ranging between six and 41 alleles, with an average of 15.85 alleles. Linkage disequilibrium was significant for 1.2% of loci pairwise LD measurement for all lakes. On the 28 sampled lakes, 16 showed significant deviation from Hardy-Weinberg equilibrium. Expected and observed heterozygosity are reported in Table S3.A5. The mean overall proportion of null alleles was 4.74%. Loci *Sfo-12* and *Sfo*C88 showed high proportion of null alleles (above 10%). Genetic analyses were performed with and without these two loci and the q-values obtained were highly correlated (Pearson, d.f. = 1359, r = 0.99 [0.994-0.995], P < 0.001). Thus, the q-values obtained with the 20 loci were used in all analyses. For several lakes (*e.g.* Boucher, Hamel, Cardinal and Pitou), all fish shared the same genetic cluster as one of the hatcheries, so they were considered as being pure domestic and a q-value = 1 was attributed to them.

Individual analyses

Body length and sex were significantly related to the infection status with the largest fish being more likely to be infected (Table 3.2a). To untangle the effect of sex, a *post hoc* test using pairwise comparisons with a Tukey adjustment for p-values was performed using the package lsmeans (Lenth, 2016). There was no difference in infection status between males and females ($P = 0.99$), but individuals with undetermined sex identification were less likely to carry parasites than males ($P = 0.003$) and females ($P = 0.006$). Body length was the only variable related to the intensity of infection, larger individuals being more likely to be in the "highly infected" group (GLMM, $n = 734$, d.f. = 730, estimate = 1.25, s.e. = 0.14, $z = 8.71$, $P < 0.001$, marginal $R^2 = 0.128$, conditional $R^2 = 0.730$).

Table 3.2 **Results of generalized linear mixed models at the individual (infection status) and population (prevalence) level and of generalized linear model at the population level (number of parasite species) in populations of *Salvelinus fontinalis***

Dependent variables	Distribution	n	d.f.	Fixed factors	Estimate	Standard error	z-value	P-value
(a) Infection status	Binomial	1240	1224	Total length	0.69	0.13	5.33	<0.001
Marginal R^2 = 0.037				Sex (indeterminate)	-1.03	0.31	-3.29	0.010
Conditional R^2 = 0.842				Sex (male)	0.02	0.20	0.08	0.933
(b) Prevalence	Binomial	28	21	Lake area	0.35	0.10	3.32	<0.001
Marginal R^2 = 0.241				Brook charr density	0.60	0.11	5.62	<0.001
Conditional R^2 = 0.382				Mean q-value	-0.67	0.09	-7.58	<0.001
				Mean total length	-0.51	0.09	-5.93	<0.001
				Mean Fulton index	0.63	0.13	4.98	<0.001
(c) Number of parasite species	Poisson	28	25	Lake area	0.20	0.10	2.06	0.039
R^2 = 0.445				Mean q-value	-0.45	0.18	-2.44	0.015

Note. Lake and wildlife reserve were used as random factors in the infection-status model and wildlife reserve was used as random factor in the prevalence model. Only variables from the final models are presented here. In the infection-status model, sex has three levels and females are the reference level. R^2 estimated with the package piecewiseSEM for GLMMs (infection status and prevalence) and R^2 of the GLM (number of parasite species) is the McFadden's pseudo-R^2.

Population analyses

All variables, except *C. commersonii* density, were significantly related to the parasite prevalence (Table 3.2b). More specifically, the proportion of infected fish was higher in larger lakes, in lakes with higher densities of *S. fontinalis* and those with the higher mean body condition of fish. However, the proportion of infected fish was lower in lakes with the smaller mean fish length and in lakes with the most domestic genetic background (*i.e.* the highest q-values, Fig. 3.2a).

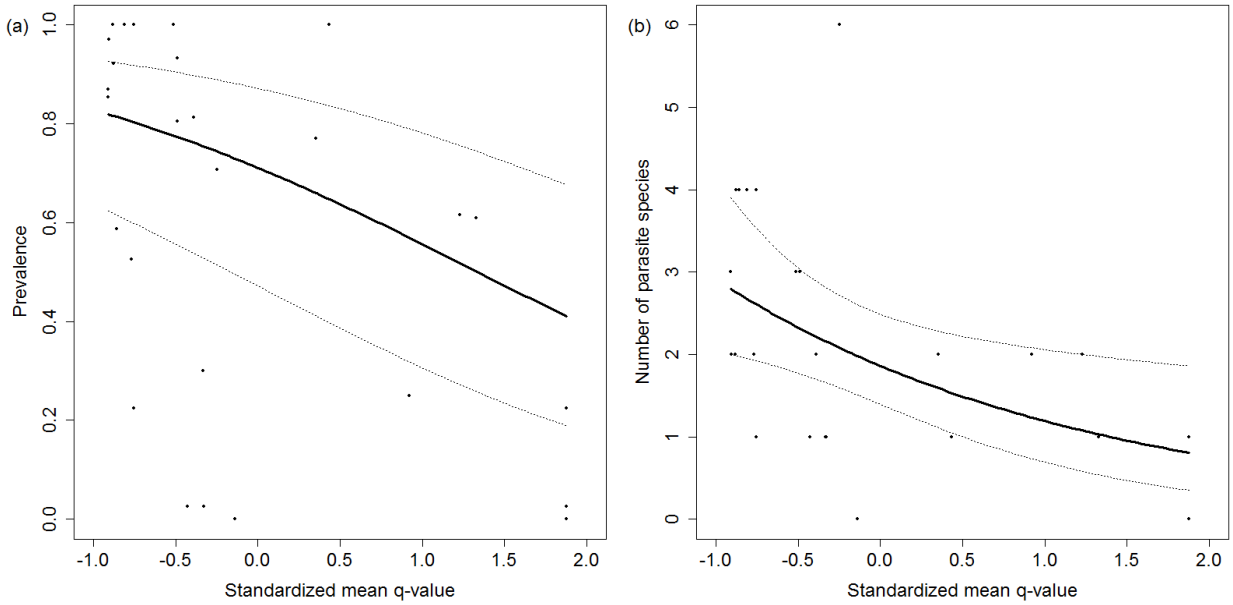

Figure 3.2 Relationships between the mean q-value (standardized, mean = 0, $\sigma^2 = 1$) of lakes and (a) parasite prevalence and (b) number of parasite species in Salvelinus fontinalis.

Full lines are the predictions of the models and dotted lines represent the 95% confidence intervals. Black dots represent raw data. The higher the q-value, the more domestic the genetic profile of the lake.

Only lake area and introgression level were related to the number of parasite species (Table 3.2c). The largest lakes displayed a higher number of parasite species, whereas a lower parasite diversity was found in lakes with higher domestic genetic backgrounds (Fig. 3.2b).

Discussion

The main objective of this study was to understand how supplementation with hatchery-reared fish and the introgression of their genes in wild populations could impact the host-parasite relationships in *S. fontinalis*. At the individual level, no evidence for an association between the genetic background and either infection status or intensity of infection was detected. Both of these variables were explained by total length, with the largest individuals being more likely to be infected. At the population level however, it appeared that lakes with a greater proportion of domestic genes displayed lower parasite prevalence and less diversified parasite communities.

Individual level

The proportion of domestic genes carried by individuals was not related to infection status or parasite load in the present study. These results are rather surprising given that resistance to parasites was shown to have a genetic basis in fishes, including in *S. fontinalis* (Glover *et al.*, 2004; Kolstad *et al.*, 2005; Perry *et al.*, 2005; Eizaguirre *et al.*, 2012) and that it was suggested that domestic fish were more susceptible to parasites. For instance, van Oosterhout *et al.* (2007) showed that after only four generations of captive breeding, guppies *Poecilia reticulata* Peters 1859 reintroduced in the wild displayed higher parasite prevalence and a higher mortality due to infection than wild fish. The results of the present study are also somewhat different from those of Currens *et al.* (1997), who showed that local adaptation was critical for parasite

resistance in *O. mykiss* and with results from Lamaze *et al.* (2014) who showed that parasite infection increased with introgression of domestic genes in *S. fontinalis*.

However, other studies suggested that hatchery-reared fish may not be more susceptible to infection than their wild counterparts. Indeed, Glover *et al.* (2004) found that among domestic and wild strains of Atlantic salmon *Salmo salar* L. 1758 exposed to sea lice *Lepeophtheirus salmonis* Krøyer 1837, the most heavily infected strain was from a wild group. Some authors thus suggested that the differences of parasite resistance observed among different fish strains were more likely to be explained by genetic differences in susceptibility (Glover *et al.*, 2004) and by local adaptation (Currens *et al.*, 1997) rather than by intrinsic differences of resistance between wild and domestic fish. Furthermore, a recent study suggested that domestic fish could actually achieve similar or even better parasite resistance than wild individuals, for instance when they come from an enriched rearing environment (*e.g.* physical structures added into the tanks, Karvonen *et al.*, 2016). Moreover, some studies have shown that domestic fish were tolerant to stress (Woodward & Strange, 1987; Solberg *et al.*, 2013), which is an important determinant of the immune response, as higher stress results in greater secretion of cortisol, a hormone with immunosuppressive effects (Bakke & Harris, 1998). A higher tolerance to stress could thus improve the capacity of domestic fish to cope with infections. Furthermore, hatchery conditions may not directly affect the parasite resistance of fish, but rather increase the pathogenicity of parasites (Suomalainen *et al.*, 2005; Pulkkinen *et al.*, 2010). This could explain the existence of parasite outbreaks in hatcheries, without implying that domestic fish are less immunocompetent. Finally, the composition of the parasites assemblage in a given study can strongly influence its results since, for instance, level of virulence can shape probability of establishment of parasites (Dobson & May, 1987), or hosts prevalence and intensity of infection (Bakke & Harris, 1998). Therefore, the number of parasite species and their characteristics such as life cycle or pathogenicity levels should be accounted for when comparing the results of different studies.

Infection status and parasitic load were mostly explained by fish length, with larger individuals being more likely to carry at least one parasite and to be more infected. Such result is common in fish studies (*e.g.* Pennycuick, 1971; Poulin *et al.*, 1991; Glover *et al.*, 2001, 2004) and is likely due to the fact that larger individuals are generally older and can have different feeding habits (Pennycuick, 1971; Hanek & Fernando, 1978; Poulin *et al.*, 1991). For instance, they can eat more and/or can feed on a wide array of different prey resulting in potentially higher probabilities of being exposed to parasites (Pennycuick, 1971). Also, older individuals likely had a longer exposure period to parasites, thus increasing their chances of getting infected and of being more infected than younger (smaller) individuals (Poulin *et al.*, 1991). Larger fish can also harbour more parasites because they present a larger contact surface (Poulin *et al.*, 1991; Glover *et al.*, 2001) and because they filter more important volumes of water through their gills (Poulin *et al.*, 1991), thus again increasing their chances of getting infected by parasites. An effect of sex on infection status was also detected with indeterminate individuals being less likely to be infected than males and females. This effect is likely explained by the fact that immature fish are smaller and younger than sexually differentiated individuals. Infection levels have been shown to vary with age in fish (Hanek & Fernando, 1978) and the most common pattern is an increase of infection with age (Pennycuick, 1971; Hanek & Fernando, 1978; Valtonen & Koskivaara, 1994), which could partly explain the pattern observed in the present study.

Population level

Prevalence and diversity of parasites were found to decrease with a greater proportion of domestic genes in lakes. This result was unexpected and somewhat contrasts with the idea that the introduction of farmed individuals into wild populations could increase the number and diversity of parasites (Wootten, 1973; van Oosterhout *et al.*, 2007; Naish *et al.*, 2008; Krkošek, 2017, but see Kennedy *et al.*, 1991; Valtonen & Koskivaara, 1994) or increase prevalence through the introduction or attraction of new hosts (McGuigan & Sommerville 1985, Dick et al.

1987, van Oosterhout et al. 2007). A lower parasitism in stocked lakes could be partly due to the higher genetic diversity that is typical of stocked *S. fontinalis* in Québec (Marie *et al.*, 2010). Indeed, a high genetic diversity is considered an important component of disease and parasite resistance (Coltman *et al.*, 1999; Eszterbauer *et al.*, 2015). Moreover, parasites can sometimes have a reduced infection success when they are confronted to non-local hosts (Voutilainen *et al.*, 2009; Kalbe *et al.*, 2016). Thus, they could be less performant in lakes where their hosts are more domestic, since they are presumably adapted to wild phenotypes. On the other hand, it is possible that the distribution of domestic individuals is a consequence of the distribution of parasites rather than the opposite. For instance, if hatchery-reared individuals are actually strongly affected by parasites (van Oosterhout *et al.*, 2007; Naish *et al.*, 2008), they could be less likely to survive in lakes with high prevalence and parasites diversity. Therefore, those lakes would display low levels of domestication because parasites limit the presence of farmed fish.

The absence of effect of introgression at the individual level and the significant association of introgression between prevalence and parasite diversity at the population level may reflect environmental differences among lakes. Lakes with the most domestic genetic background are the most heavily stocked in the system studied here (Marie et al., 2010; Létourneau et al., 2018). It has been suggested that intense stocking could alter environmental quality of habitats, for instance by altering zooplankton community structure and thus food resources (*e.g.* Mcnaught *et al.*, 1999; Yang *et al.*, 2005). Therefore, a more domestic genetic background could be confounded with poor quality environments, which could in turn influence the presence of parasites in lakes. Moreover, typically, intensively stocked lakes are supplemented because they are generally poorer environments in which populations are less productive (SÉPAQ, personal communication), and therefore would not sustain angling pressures without external supply. In the present study system, the presence of lakes with only pure domestic individuals suggests that either wild stocks have been replaced by domestic fish because of stocking (Evans & Willox, 1991) or that wild populations never managed to settle in these lakes in the first place, possibly because of poor environmental quality. In the present study, this possibility could not be tested because no detailed data on abiotic conditions (besides lake area which was positively

related to prevalence and number of parasite species) were available in these lakes. Also, since lakes are supplemented to increase angling success, heavily stocked lakes are more likely to be exposed to stronger angling pressure. Those lakes could have depleted parasite communities because of the process of "fishing out parasites" through host density reduction, a phenomenon already described in marine systems in which the massive removal of hosts trough fishing leads to a global decline in parasites (Dobson & May, 1987; Wood *et al.*, 2010; Krkošek, 2017). Furthermore, largest individuals that carry more parasites are also preferentially targeted by recreational anglers. Thus, it is possible that the removal of largest individuals in lakes that sustain important fishing pressures (*i.e.* heavily stocked lakes) lead to depleted parasites communities in these populations.

In conclusion, to our knowledge, no previous work addressed the consequences of introgressive hybridization of domestic fish on their wild conspecifics by documenting both individual and population measures of parasitism. The present results show no effect of the domestic genes at the individual level. At the population level, most introgressed populations are characterized by the occurrence of fewer parasites, but this could partly be explained by confounding environmental effects. To disentangle the effects of genetics and environment on the parasitism patterns of stocked lakes, additional environmental variables should be analysed, such as lake depth which is known to influence parasite communities in some cases (Marcogliese & Cone, 1991; Dubois *et al.*, 1996; Bergeron *et al.*, 1997).

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CHAPITRE 4
TAILLE EFFECTIVE

Description de l'article et contribution

La taille effective est un paramètre essentiel pour la persistance des populations et le maintien de leur diversité génétique. Bien que les ensemencements augmentent le nombre total d'individus des populations, leurs effets sur la taille effective peuvent être négatifs. Cependant, peu d'études empiriques analysent le lien entre les ensemencements et la taille effective, particulièrement lorsque les ensemencements s'effectuent sur de longues périodes de temps. De plus, peu d'informations existent sur l'importance de l'intensité des ensemencements en lien avec la taille effective. Le but de cet article était donc, dans un premier temps, de déterminer comment les ensemencements en tant que tels affectent les tailles effectives des populations. Par la suite, un second objectif était de déterminer comment l'intensité des ensemencements pouvait moduler ces effets. Nos résultats montrent que les lacs ensemencés ont des tailles effectives significativement plus faibles que les lacs n'ayant jamais été supplémentés. Cependant, l'intensité d'ensemencement évaluée ici selon différents critères ne semble ici avoir qu'un effet négligeable sur les tailles effectives. Ainsi, les tailles effectives plus faibles des lacs ensemencés sont probablement imputables à des effets environnementaux plutôt qu'à des effets directs des ensemencements.

Pour cet article, j'ai participé à la collecte des données (2015-2016). Je tiens à remercier Raphaëlle Dubois et Nicolas Bousquet pour leur aide concernant le travail de terrain et de laboratoire. L'élaboration des idées s'est faite en collaboration avec Dany Garant. J'ai analysé les données et rédigé le manuscrit. Dany Garant a supervisé les analyses et révisé plusieurs versions du manuscrit. Louis Bernatchez a révisé le manuscrit et apporté de nouvelles pistes d'interprétation. Pascal Sirois a révisé le manuscrit.

Impacts of stocking and its intensity on effective population size in Brook Charr (*Salvelinus fontinalis*) populations

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Abstract

Effective population size (Ne) is a measure of the genetic size of a population and a crucial parameter for wildlife population management since it is strongly related to retention of genetic diversity in time and/or to inbreeding levels. Many exploited fish populations are stocked with the purpose of increasing population sizes to sustain important fishing pressures. However, stocking hatchery-reared fish could at the same time increase population census size and decrease Ne. Our study aimed at characterizing how stocking affected Ne in supplemented populations of Brook Charr (*Salvelinus fontinalis*) in Québec and at assessing how this relationship varied with the intensity of stocking (e.g. number of stocking events, number of fish stocked/ha, proportion of domestic genetic background). We estimated Ne with the linkage disequilibrium method in 54 populations (3361 sampled individuals analyzed at 20 microsatellites) with various levels of stocking intensity. We found that stocked lakes have significantly lower Ne than unstocked lakes. However, we found little evidence of an additional effect of stocking intensity on Ne of stocked lakes. Our results suggest that stocking may have a negative impact on Ne but that more intense stocking does not necessarily translate into lower Ne. However, even though low Ne in stocked populations could be attributed to an effect of stocking, it is also likely that stocked lakes consist of poor environments that translate into low Ne.

Keywords: Effective size; Hatchery supplementation; Linkage disequilibrium method; Salmonids; Microsatellites

Introduction

Estimating population sizes accurately is an important part of wildlife conservation and management, and different estimators are used with a distinction between counts of individuals (e.g. census size, number of breeders) and genetic size of populations (Hamilton 2009). The latter, also called effective population size (Ne), is defined as the size of an ideal and theoretical population losing its genetic variability at the same rate as the studied population (Wright 1931). Ne is influenced by various factors such as sex-ratio or age structure (Charlesworth 2009) and is mostly affected by the demographic history of the studied population and variance in reproductive success among individuals (Frankham 1995; Ardren and Kapuscinski 2003; Araki et al. 2007b; Ruzzante et al. 2016). Typically, Ne is in the order of 10%-20% of census size (Frankham 1995; Palstra and Fraser 2012) and can be much lower, for instance in marine species (Hedgecock 1994). This is due to the fact that characteristics of natural populations deviate from those of an ideal population (Frankham et al. 2002, 2004). Obtaining reliable estimates of Ne is of particular interest not only for wildlife management (e.g. Saarman et al. 2017), but also for conservation biology (Palstra and Ruzzante 2008; Frankham et al. 2014). Indeed, Ne is closely linked to crucial genetic features that determine long-term population viability, such as inbreeding, genetic drift or maintenance of genetic variation across generations (Frankham 1996; Frankham et al. 2014). Ne is therefore an important parameter to monitor in exploited or endangered populations.

Populations at risk are frequently the target of management actions that can influence Ne (Tringali and Bert 1998; Lorenzen et al. 2012). For instance, stocking exploited or endangered wild populations with individuals produced in captivity is a widespread conservation practice, with the objective of attaining or maintaining sustainable population sizes (Brown and Day 2002; Frankham et al. 2002; Laikre et al. 2010; Soorae 2013). However, even though the most direct consequence of stocking is an increase of census size, it can also, at the same time, decrease Ne under certain circumstances. For instance, supportive breeding could be detrimental

to Ne because it favors the reproduction of a small number of individuals, which may substantially increase variance in reproductive success among individuals and thus reduce Ne (Ryman and Laikre 1991). Previous studies have also showed that Ne can be depleted after stocking through the increase of variance in reproductive success because of the introduction of individuals with a poor fitness (Araki et al. 2007b) as it is often the case with fish produced by hatcheries (Araki et al. 2007a, b, 2008; Araki and Schmid 2010; Christie et al. 2012b, 2014).

In fishes, stocking intensity can vary greatly depending on the stocked population and on the goal of the supplementation program. The number of stocked individuals is thus often highly variable and may represent a more or less important proportion of the stocked population. Furthermore, the number of times populations are supplemented can also be variable and range from a single to dozens stocking events. It is however unclear how the intensity and frequency of stocking affect Ne. It has been suggested that, in cases of supportive breeding (i.e. local strains kept a single or a few generations in hatcheries), the depletion of Ne is stronger with an increase of the relative contribution of hatchery-reared fish (Ryman and Laikre 1991; Tringali and Bert 1998; Christie et al. 2012a). However, Araki et al. (2007b) failed to detect a negative effect of supportive breeding on Ne, even though they showed a depletion of Ne when stocked fish originated from traditional hatcheries (i.e. non-local strains kept for several generations in hatcheries). It is also unknown how Ne adjusts once stocking has stopped. Since Ne depletion after stocking is attributable to an increase in variance in reproductive success, whether it is due to the artificial breeding of a small portion of the population (Ryman and Laikre 1991) or to the presence of domestic individuals with a low fitness (Araki et al. 2007b), the removal of stocked individuals should restore Ne. Considering that, in the case of the introduction of non-local individuals, the proportion of domestic genetic background reflects the presence of hatchery-reared individuals, and that it can decrease after the interruption of stocking (Hansen et al. 2009; Harbicht et al. 2014; Valiquette et al. 2014; Létourneau et al. 2018; White et al. 2018), it is plausible that stopping supplementation could increase Ne over time.

Stocking is largely used to supplement fish populations (Brown and Day 2002), especially for salmonids (Araki and Schmid 2010), given their considerable economic and recreative importance and the global decline of their populations (Brown and Day 2002; Post et al. 2002). Yet, stocking practices may result in additional problems for these species. For instance, the diminution of Ne in populations of Brown trout (*Salmo trutta*) in Europe, after decades of introgression of domestic genes in wild populations, raised questions about the conservation implications of stocking in salmonids (Hansen et al. 2009). In North America, Brook Charr (*Salvelinus fontinalis*) is a popular species for recreational angling and it has been widely stocked both inside and outside its native range. In particular, it is the most intensively stocked species in the province of Québec, Canada, representing over 80% of the biomass of stocked fish in 2016 (i.e. over 20 tons of fish, Ministère des Forêts, de la Faune et des Parcs). Previous studies showed that stocking lakes with domestic Brook Charr affects the genetic makeup of populations by increasing genetic diversity and homogenizing genetic structure among populations, with stronger effects in heavily stocked lakes (Marie et al. 2010; Lamaze et al. 2012). In this context, quantifying the impacts of stocking intensity on Ne may help to uncover the consequences of supplementation measures and improve their effectiveness and the viability of populations in the long-term.

In our study, we used microsatellites and data collected from 42 lakes over 2 time periods in wildlife reserves in Québec with various stocking histories, ranging from pure wild lakes to lakes intensively stocked, to assess the impacts of stocking on Ne in Brook Charr. Stocked fish do not originate from stocked lakes, and it has already been shown in our system that intensively stocked lakes display higher levels of introgression of exogenous genes (Marie et al. 2012). Since the potential negative influence of stocking on Ne is linked to a greater variance in reproductive success (Ryman and Laikre 1991; Araki et al. 2007b), and given that domestic individuals generally have a lower reproductive success (Araki et al. 2007a, 2008), we predict that stocked lakes will show lower Ne than unstocked ones. We also expect that, within stocked lakes, Ne will decrease as a function of stocking intensity and genetic introgression will result in lower Ne. Finally, we predict that effects of stocking on Ne will not be permanent and that

they will decrease when stocking stops, since domestic genes are often purged from populations when stocking stops in this species (Létourneau et al. 2018; White et al. 2018; but see Harbicht et al. 2014).

Methods

Sampling sites and procedures

We conducted sampling in three wildlife reserves (Portneuf [47°10'17.8"N, 72°20'32.7"W], Mastigouche [46°42'45.2"N, 73°25'37.7"W] and Saint-Maurice [47°04'00.0"N, 73°08'28.5"W]) in Québec, Canada over two time periods: 2007-2008 and 2014-2016. Overall, 42 lakes were sampled, 12 of them during both sampling periods, resulting in 54 populations (i.e. unique lake-period combinations). The stocking history of those lakes have been recorded since 1964 (provided by the Ministère des Forêts, de la Faune et des Parcs) for the following parameters: how many times each lake has been stocked, during which years and with how many fish. Some of the sampled lakes were stocked intensively for decades while others were not stocked for years or were never stocked (Table 4.1). Stocked fish originated from different hatcheries using different breeding methods. Fish from Jacques-Cartier hatchery were kept in captivity from multiple generations and used to supplement lakes of the Portneuf reserve. Mastigouche and Saint-Maurice reserves used fish from Lac-des-Écorces and Saint-Alexis-des-Monts hatcheries which breed hybrid strains by crossing domestic fish and wild fish from Lake Bourassa, located in the Mastigouche reserve. Fish are generally stocked at early life stages such as fry.

We captured between 32 and 138 fish per lake-period combination (mean = 62, SD = 25, Table 4.1) with gill nets and for a total of 3 361 sampled individuals. All cohorts were sampled indiscriminately, resulting in a wide range of sizes (and thus ages) for each lake (Fig. S4.A1 and

S4.A2). All lakes were sampled before the stocking events of the sampling year to avoid the capture of recently stocked individuals. After each capture, we collected the adipose fin of each fish and preserved it in 95% ethanol for later DNA extraction. All protocols and procedures employed were reviewed and approved by the Ministère des Forêts, de la Faune et des Parcs. Moreover, we obtained samples from hatcheries (Jacques Cartier "JC", n = 53, Saint-Alexis des Monts "A", n = 80, Lac des Écorces "ECO", n = 40) and from lake Bourassa ("BOU", n = 40).

Genetic analyses

We extracted DNA from adipose fin clips. Details of DNA extraction, PCR amplification and genotyping are described in Gossieaux et al. (2018). We genotyped samples from the 2007-2008 sampling period at 19 microsatellite loci and samples from 2014-2016 period at 20 loci (Table S4.A1). We checked genetic data for genotyping errors and calculated error rates as described in Bonin et al. (2004). We used Genepop v. 4.3 (Rousset 2008) to assess Hardy-Weinberg equilibrium and linkage disequilibrium (LD) with a Bonferroni correction to account for multiple testing. We estimated allelic richness and checked for the presence of null alleles for each locus with Cervus v. 3.0.7 (Kalinowski et al. 2007). We computed expected and observed heterozygosity with the package 'adegenet' (Jombart and Ahmed 2011) in R.

We estimated the proportion of domestic genetic background in each population by estimating the mean introgression level. To do so, we first estimated the introgression level (q-value) of each individual with the software Structure v. 2.3.4 (Pritchard et al. 2000) with a 100 000 burn-in period and 250 000 Markov Chain Monte Carlo iterations. We used the web service Structure Harvester (Earl and VonHoldt 2012) and the software CLUMPP (Jakobsson and Rosenberg 2007) to process the Structure outputs. We analyzed the individuals of each sampled population by comparing them to the hatchery samples used to stock the given lake. We fixed the number of genetic populations (K) according to the probable number of populations in each lake

considering the number of strains used for stocking (K=2 for lakes of the Portneuf reserve, K=4 for lakes of both the Mastigouche and Saint-Maurice reserves). The individual introgression level varied between 0 and 1, respectively designating pure wild and pure domestic individuals. We then calculated the mean introgression level of all the individuals of each population to obtain an estimate of the proportion of domestic genes.

Table 4.1 Characteristics of all hatcheries and sampled populations including their stocking history, genetic features and effective population sizes (Ne_{LD}, estimated with the linkage disequilibrium method).

Origin	Lake	Population	n	Lake area (ha)	Number of stocking events	Number of fish stocked/ha	Number of years since last stocking	Proportion of domestic genes*	Ne_{LD} [95% CI]	He	Ho
MAS	Demarest	07-08_DEM	138	5.2	1	192	9	1.0%	14.7 [11.2-18.8]	0.58	0.57
MAS	Petit Saint-Bernard	07-08_PET	55	14.3	2	559	4	20.9%	29.0 [24.4-35.0]	0.70	0.65
MAS	Mercure	07-08_MER	82	3.1	2	471	2	39.3%	31.4 [26.1-38.1]	0.74	0.73
MAS	Gélinotte	07-08_GEL	72	4.7	6	1194	0	55.0%	32.7 [26.1-41.5]	0.67	0.75
MAS	Brochard	07-08_BROCH	71	15.5	17	2870	1	50.8%	34.0 [27.4-42.8]	0.67	0.71
MAS	Arlequin	07-08_ARL	84	6.5	0	0	NA	0.9%	40.5 [27.1-64.5]	0.33	0.30
MAS	Pitou	07-08_PIT	101	8.0	19	2003	1	61.1%	57.0 [44.1-76.1]	0.70	0.78
MAS	Deux Étapes	07-08_DETP	89	12.3	19	3261	1	50.1%	74.3 [61.0-92.5]	0.68	0.76
MAS	Hollis	07-08_HOL	82	16.7	18	2220	1	38.8%	79.0 [62.0-104.7]	0.68	0.79
MAS	Head	07-08_HEAD	74	9.7	1	258	36	4.8%	79.3 [55.7-124.7]	0.64	0.64
MAS	Moyen	07-08_MOY	93	19.2	0	0	NA	0.7%	84.6 [37.0-504.1]	0.35	0.39
MAS	Chamberlain	07-08_CHAMB	106	18.4	9	938	2	30.1%	124.5 [96.3-169.5]	0.76	0.75
MAS	Cerné	07-08_CER	108	13.2	0	0	NA	4.4%	181.0 [103.0-492.0]	0.58	0.56
PN	Amanites	07-08_AMA	84	8.0	26	778	0	54.5%	2.9 [2.6-3.1]	0.69	0.57
PN	Caribou	07-08_CAR	77	5.0	1	40	0	18.9%	8.8 [7.2-10.5]	0.62	0.58
PN	Belles-de-jour	07-08_BEL	76	5.0	27	2277	0	55.3%	13.4 [11.9-15.0]	0.73	0.66
PN	Veillette	07-08_VEI	98	36.0	8	259	3	0.6%	18.2 [15.5-21.2]	0.61	0.59
PN	Rivard	07-08_RIV	47	5.0	8	1438	22	0.9%	22.6 [18.6-27.9]	0.61	0.61
PN	Méthot	07-08_MET	100	8.0	22	3951	0	30.2%	36.9 [32.2-42.5]	0.75	0.71

Origin	Lake	Population	n	Lake area (ha)	Number of stocking events	Number of fish stocked/ha	Number of years since last stocking	Proportion of domestic genes*	Ne_{LD} [95% CI]	He	Ho
PN	Arcand	07-08_ARC	57	16.0	1	78	35	1.9%	41.6 [29.5-62.9]	0.50	0.50
PN	Langoumois	07-08_LANG	38	10.0	0	0	NA	0.5%	59.2 [37.0-119.9]	0.58	0.52
PN	Sorbier	07-08_SOR	83	5.0	0	0	NA	1.0%	99.4 [69.4-159.3]	0.60	0.56
PN	Circulaire	07-08_CIR	70	0.9	2	6111	10	2.5%	105.6 [71.5-181.7]	0.52	0.53
PN	Main de Fer	07-08_MDF	115	16.0	0	0	NA	0.4%	2503.0 [282.4-10 000.0]	0.47	0.48
MAS	Deux Étapes	14-16_DETP	40	12.3	22	3647	3	28.3%	42.3 [31.8-59.5]	0.68	0.81
MAS	Demarest	14-16_DEM	66	5.2	1	192	16	1.2%	50.8 [30.7-100.7]	0.58	0.57
MAS	Chamberlain	14-16_CHAMB	41	18.4	9	938	9	15.9%	54.4 [42.4-73.4]	0.74	0.74
MAS	Pitou	14-16_PIT	40	8.0	23	2621	2	100.0%	58.9 [43.3-87.3]	0.70	0.82
MAS	Head	14-16_HEAD	46	9.7	1	258	43	4.6%	87.8 [58.2-160.1]	0.63	0.64
MAS	Cerné	14-16_CER	67	13.2	0	0	NA	6.4%	151.9 [85.6-456.6]	0.58	0.57
PN	Amanites	14-16_AMA	71	8.0	35	1137	2	19.4%	3.9 [3.5-5.2]	0.58	0.51
PN	Caribou	14-16_CAR	32	5.0	11	570	2	19.5%	20.8 [16.0-27.8]	0.64	0.62
PN	Méthot	14-16_MET	50	8.0	38	4661	1	28.3%	35.8 [30.5-42.4]	0.74	0.70
PN	Langoumois	14-16_LANG	41	10.0	0	0	NA	1.0%	91.1 [49.7-308.2]	0.56	0.52
PN	Sorbier	14-16_SOR	46	5.0	0	0	NA	0.9%	377.9 [131.4-10 000.0]	0.60	0.57
PN	Main de Fer	14-16_MDF	38	16.0	0	0	NA	2.2%	538.4 [92.9-10 000.0]	0.45	0.46
STM	Est	14-16_EST	40	12.2	14	2246	1	45.9%	12.6 [10.4-15.3]	0.68	0.69
STM	Bec-Scie	14-16_BEC	41	9.7	20	2974	1	66.1%	20.5 [17.4-24.4]	0.69	0.73
STM	Pin	14-16_PIN	64	5.9	13	2525	1	80.5%	26.9 [23.4-31.2]	0.72	0.78
STM	Clairval	14-16_CLAI	40	24.9	21	2474	1	15.1%	30.8 [23.9-41.0]	0.64	0.66
STM	Plongeon-Huard	14-16_PLON	40	5.3	16	2817	1	21.5%	31.4 [24.6-41.5]	0.68	0.76

Origin	Lake	Population	n	Lake area (ha)	Number of stocking events	Number of fish stocked/ha	Number of years since last stocking	Proportion of domestic genes*	Ne_{LD} [95% CI]	He	Ho
STM	Sud	14-16_SUD	40	6.4	18	2948	1	77.1%	36.7 [29.0-48.2]	0.71	0.71
STM	Corbeil	14-16_CORB	43	9.5	2	526	44	2.0%	39.9 [26.7-67.1]	0.60	0.58
STM	Courbé	14-16_COUR	40	105.1	22	2083	1	6.0%	42.2 [30.6-63.0]	0.66	0.82
STM	Hamel	14-16_HAM	43	10.7	31	6532	1	100.0%	48.8 [37.4-67.2]	0.68	0.80
STM	Soucis	14-16_SOU	41	267.5	1	2804	39	24.6%	54.9 [41.4-77.4]	0.69	0.66
STM	Cardinal	14-16_CARD	40	7.1	24	8130	2	100.0%	57.2 [43.1-81.4]	0.71	0.77
STM	Boucher	14-16_BOUCH	40	25.1	34	5420	2	100.0%	62.6 [44.5-98.3]	0.70	0.79
STM	Ecarté	14-16_ECAR	41	6.3	18	4359	2	21.7%	66.2 [46.5-106.4]	0.70	0.72
STM	Epervier	14-16_EPER	40	12.8	9	1164	1	18.1%	80.6 [51.2-162.4]	0.66	0.79
STM	Perdu	14-16_PER	43	22.1	16	2293	25	48.7%	86.8 [54.3-182.5]	0.64	0.65
STM	Brown	14-16_BRO	63	273.0	4	114	40	2.7%	94.0 [66.0-150.5]	0.65	0.65
STM	Portage	14-16_PORT	40	46.9	11	1044	25	6.5%	153.4 [74.5-2043.4]	0.51	0.53
STM	Milord	14-16_MIL	44	46.7	16	1175	10	15.9%	2198.1 [261.0-10 000.0]	0.71	0.70
Hatchery	Lac des Écorces	07-08_ECO	40	NA	NA	NA	NA	NA	53.6 [35.2-96.4]	0.65	0.64
Hatchery	Saint-Alexis	07-08_A	40	NA	NA	NA	NA	NA	83.4 [58.0-138.4]	0.70	0.79
Hatchery	Jacques Cartier	07-08_JC	53	NA	NA	NA	NA	NA	178.5 [112.9-383.6]	0.73	0.71
Hatchery	Saint-Alexis	14-16_A	40	NA	NA	NA	NA	NA	51.5 [37.8-75.9]	0.70	0.78

Abbreviations: STM = Saint-Maurice, MAS = Mastigouche, PN = Portneuf, n = number of sampled individuals, Ne_{LD} [95% CI] = effective population size calculated with the linkage disequilibrium method (PCrit = 0.05) with 95% confidence intervals estimated with the jackknife method, He = expected heterozygosity, Ho = observed heterozygosity. “NA” is for non-available data.

* Proportion of domestic genes = mean q-value x 100

Ne estimates

We estimated Ne with the bias-corrected linkage disequilibrium method (Ne_{LD}, Hill 1981) developed by (Waples and Do 2008) using 20 microsatellite loci (Table S4.A1). To do so, we used the software NeEstimator v.2.01 (Do et al. 2014) assuming random mating (for an overview of the assumptions of this method and the possible bias, see the part 4.4. of our Discussion - *Estimating Ne in natural contexts*). We used two thresholds of rare alleles exclusion (allelic frequency less than 0.02 and 0.05) since their presence can bias Ne estimations (Waples and Do 2010). For the 54 populations, NeEstimator produced a single estimation of infinite Ne (i.e. lake 07-08_MDF). This happens when the genetic results can be totally explained by sampling error rather than by genetic drift and, in this situation, it can be concluded that the population is "very large" (Waples and Do 2010). To account for this large population in our analyses, we attributed to it an effective population size calculated as:

$$Ne_{inf} = Ne_{max} + sd_{Ne}$$

with Ne_{inf} the value used as replacement of the infinite estimate, Ne_{max} the highest Ne estimate we obtained and sd_{Ne} the standard deviation of all Ne estimates. Therefore, it was the highest Ne of our samples but it was close to the range of the other estimated values (Table 4.1). We also obtained infinite estimates for the higher confidence intervals for four populations. We attributed a fixed value of 10 000 to these infinite estimates in order to keep these populations in our analyses while keeping very large confidence interval estimates (Table 4.1). To further assess the influence of these values on our results, all analyses were performed with and without them.

Since we sampled 12 of our lakes during both of the time periods, we also estimated their Ne with the temporal method (Ne_{TM}, Krimbas and Tsakas 1971) with NeEstimator, using again two thresholds of rare alleles exclusion (0.02 and 0.05). We considered that our sampling periods were two generations apart (generation time is two to three years in Brook Charr, e.g. COSEWIC, 2000; Kazyak et al. 2016; Ruzzante et al. 2016) and we used the three possible estimation methods implemented in NeEstimator: Pollack, Nei-Tajima and Jorde-Ryman. Since these methods gave very similar results (Table S4.A2), we only present the results from the Pollack method hereafter named Ne_{TM}. Finally, we also estimated Ne with the sibship assignment method ($Ne_{SIB,}$ Wang 2009) with the software Colony v.2.0.6.4 (Jones and Wang 2010), assuming non-random mating given that most of our lakes are not at the Hardy-Weinberg equilibrium (see section "3. Results"). We assessed similarity among these three different estimates ($Ne_{LD,}$ $Ne_{TM,}$ Ne_{SIB}) with Pearson correlations. The sibship method is however sensitive to sample size, since its accuracy relies on the proportion of the population that is sampled (Wang 2009; Johnstone et al. 2012), and tends to underestimate Ne in large populations when sampling effort is limited (e.g. DeFaveri and Merilä 2015; Ferchaud et al. 2016). To account for this bias, we excluded the 10% of populations with the largest Ne_{LD} and Ne_{TM} estimates to compare them to Ne_{SIB}.

Effects of stocking and its intensity

To assess whether stocking affects Ne, we assigned to each population a status of "unstocked" (i.e. populations that were never stocked, n = 10) or "stocked" (i.e. populations that were stocked at least once, n = 44). We used Ne as a response variable in a generalized linear model (GLM) with a negative binomial distribution to account for overdispersion. We included stocking status, lake area in hectares (available in the database provided by the Ministère des Forêts, de la Faune et des Parcs) and wildlife reserve identity (to account for other potential unmeasured differences among reserves, for instance in terms of stocking management) as fixed effects. No other environmental variables were available for all of our populations.

The effect of stocking intensity on Ne was assessed using the following stocking variables: i) number of times a population was stocked, ii) overall number of fish stocked per hectare, iii) proportion of domestic genes and iv) number of years since last stocking event. We only considered stocked populations (n = 44) and used Ne as a response variable in all our analyses. We used GLMs with a negative binomial distribution. We always included lake area and the wildlife reserve identity as fixed effects. All the variables linked to intensity of stocking were not independent from each other. Indeed, the proportion of domestic genetic background in our system has been previously shown to be significantly explained by the number of years since the mean stocking year and by the interaction between number of stocking events and number of fish stocked per hectare (Létourneau et al. 2018). Moreover, all these variables are significantly correlated with each other (Table S4.A3). To avoid multicollinearity, we thus analyzed them separately. We therefore built four separate models with different independent variables: (1) the number of stocking events, (2) the number of fish stocked/ha, and (3) the proportion of domestic genetic background to determine how stocking and its intensity affect Ne; we also used (4) the number of years since last stocking event to determine how Ne changes when stocking stops.

We simplified all models with a backward stepwise model selection approach using likelihood ratio tests ($\alpha = 0.05$). We standardized all continuous independent variables (mean = 0, variance = 1). In order to avoid multicollinearity, we checked the variance inflation factor for all models (VIF<3; Graham, 2003). We performed all our analyses in R v.3.4.3 and we used the packages MASS to fit GLMs (Venables and Ripley 2002) and piecewiseSEM to calculate R^2 (Lefcheck 2016).

Results

All loci were polymorphic and allelic richness ranged between 8 and 48 alleles with an average of 18.8 alleles. Mean genotyping error rate varied between 0% (for 11 loci) and 2.5% with a mean of 0.6%. From our 54 populations, 39 showed significant deviation from Hardy-Weinberg equilibrium. Linkage disequilibrium was significant for 7.2% of loci pairwise LD measurement. More specifically, three populations (07-08_AMA, 07-08_CAR, 14-16_AMA) had over 45% of their loci in LD. When they were removed, the global LD dropped to 4.4%. All analyses were thus performed with and without these populations to make sure that they did not influence our results. Expected and observed heterozygosity are reported in Table 4.1. Locus *Sfo-12* displayed a significantly higher percentage of null alleles than other loci (27%). However, we previously showed that excluding this locus from analyses does not change the estimation of q-values (Gossieaux et al. 2018) and we therefore kept it in our analyses. In four populations (14-16_BOUCH, 14-16_HAM, 14-16_CARD and 14-16_PIT), all fish shared the same genetic cluster as one of the hatcheries, indicating that they were pure domestic. We thus attributed them a proportion of domestic genes of 1.

Ne estimations using either 0.02 or 0.05 thresholds of rare alleles exclusion were highly correlated for Ne_{LD} (Pearson, df = 52, r = 0.95 [0.91-0.97], $p < 0.01$) and Ne_{TM} (Pearson, df = 10, r = 0.78 [0.36-0.93], $p < 0.01$). Therefore, we only present here the results of the analyses performed with estimators calculated with the exclusion threshold of 0.05 since it is considered to be more conservative and least biased (Waples and Do 2010; Nunziata and Weisrock 2018). Ne estimates obtained with all methods with both thresholds are available in Table S4.A4. Results of our statistical analyses using Ne_{LD} with a 0.02 threshold are also provided in supplementary material (Tables S4.A5 and S4.A6).

The similarity of the Ne estimates obtained with the different methods (Ne_{LD}, Ne_{TM}, Ne_{SIB}) was equivocal. Ne_{TM} was not correlated to Ne_{LD} (Pearson, df = 10, r = 0.40 [-0.23 – 0.79], $p = 0.20$) nor Ne_{SIB} (Pearson, df = 8, r = 0.57 [-0.09 – 0.89], $p = 0.57$), whereas Ne_{SIB} was significantly correlated to Ne_{LD} (Pearson, df = 46, r = 0.63 [0.42 – 0.78], $p < 0.01$). Since Ne_{TM} was only estimated for 12 lakes and Ne_{SIB} is less reliable for large populations, we only used Ne_{LD} for further analyses.

We found a significant effect of stocking status on Ne_{LD} with unstocked populations having significantly higher Ne_{LD} (unstocked populations average Ne_{LD} = 707 [343-1458]) than stocked populations (stocked populations average Ne_{LD} = 69 [50-96]; Table 4.2). Ne was however not influenced by lake area and, among all populations, those of the Saint-Maurice reserve displayed significantly higher Ne_{LD} than those from Portneuf and Mastigouche reserves (Table 4.2). The final model containing stocking status and reserve identity explained 36.6% of the total variance in Ne_{LD} (Table 4.2) but 24.9% of total variance in Ne_{LD} was explained by stocking status alone.

Table 4.2 Final model (GLM, negative binomial distribution) assessing whether stocking status impacts effective population size (Ne_{LD} estimated with the linkage disequilibrium method, PCrit=0.05).

Dependent variable	n	Fixed factors	Estimate	SE	z-value	*p*
Ne_{LD}	54	Intercept	5.16	0.25	20.39	< 0.001
$R^2 = 0.366$		Reserve Mastigouche	-1.31	0.36	3.60	< 0.001
		Reserve Portneuf	-1.49	0.39	3.79	< 0.001
		Stocking status Unstocked	2.33	0.41	5.74	< 0.001
		Variable removed:	Lake area (p = 0.752)			

In all stocking intensity models, wildlife reserve identity was always significant with a general pattern of lakes from the Portneuf reserve having the lowest Ne_{LD} and Saint-Maurice reserve the highest (see Table 4.3 for details). Again, Ne_{LD} was not influenced by lake area (all $p > 0.05$). Similarly, the number of stocking events and number of fish stocked per hectare were not related to Ne_{LD} (Table 4.3, Fig. 4.1a, b). However, the proportion of domestic genetic background (i.e. mean q-value) was significantly negatively related to Ne_{LD} (Table 4.3, Fig. 4.1c). The final model that included both the proportion of domestic genetic background and reserve identity explained 40.1% of total variance in Ne_{LD} and 10.0% of total variance in Ne_{LD} was explained by the proportion of domestic genetic background only. Finally, the number of years since last stocking had no impact on Ne_{LD} (Table 4.3, Fig. 4.1d), suggesting that Ne_{LD} did not change after interruption of stocking activity.

Table 4.3 Final models (GLM, negative binomial distribution) assessing whether stocking intensity is related to effective population size (Ne_{LD} estimated with the linkage disequilibrium method, PCrit=0.05).

Dependent variable	n	Fixed factors	Estimate	SE	z-value	*p*
Final model with non-significant intensity of stocking variables						
Ne_{LD}	44	Intercept	4.04	0.27	15.24	<0.001
R^2=0.302		Reserve Mastigouche	-1.13	0.36	-3.15	0.002
		Reserve Portneuf	-1.82	0.39	-4.62	<0.001
		Variables removed:	Lake area ($p \geq 0.64$)			
			Number of stocking events ($p = 0.38$)			
			Number of fish stocked/ha ($p = 0.55$)			
			Number of years since last stocking ($p = 0.34$)			
	43		Mean q-value without lake 14-16_MIL ($p = 0.097$)*			
Final model with significant proportion of domestic background (mean q-value)						
Ne_{LD}	44	Intercept	4.12	0.25	16.62	<0.001
R^2=0.401		Reserve Mastigouche	-0.95	0.34	-2.82	0.005
		Reserve Portneuf	-2.01	0.38	-5.24	<0.001
		Mean q-value	-0.47	0.15	-3.08	0.002
		Variable removed:	Lake area ($p = 0.59$)			

Dependent variable	n	Fixed factors	Estimate	SE	z-value	*p*
Final model with significant number of years since last stocking without lake 14-16_MIL						
Ne_{LD}	43	Intercept	3.95	0.14	27.46	< 0.001
$R^2 = 0.254$		Reserve Mastigouche	0.09	0.21	0.41	0.68
		Reserve Portneuf	-0.61	0.23	-2.60	0.009
		Number of years since last stocking without lake 14-16_MIL	0.23	0.09	2.50	0.012
		Variable removed:	Lake area (p = 0.89)			

* When the population 14-16_MIL was removed, mean q-value was only marginally non-significant

R^2 are from final models.

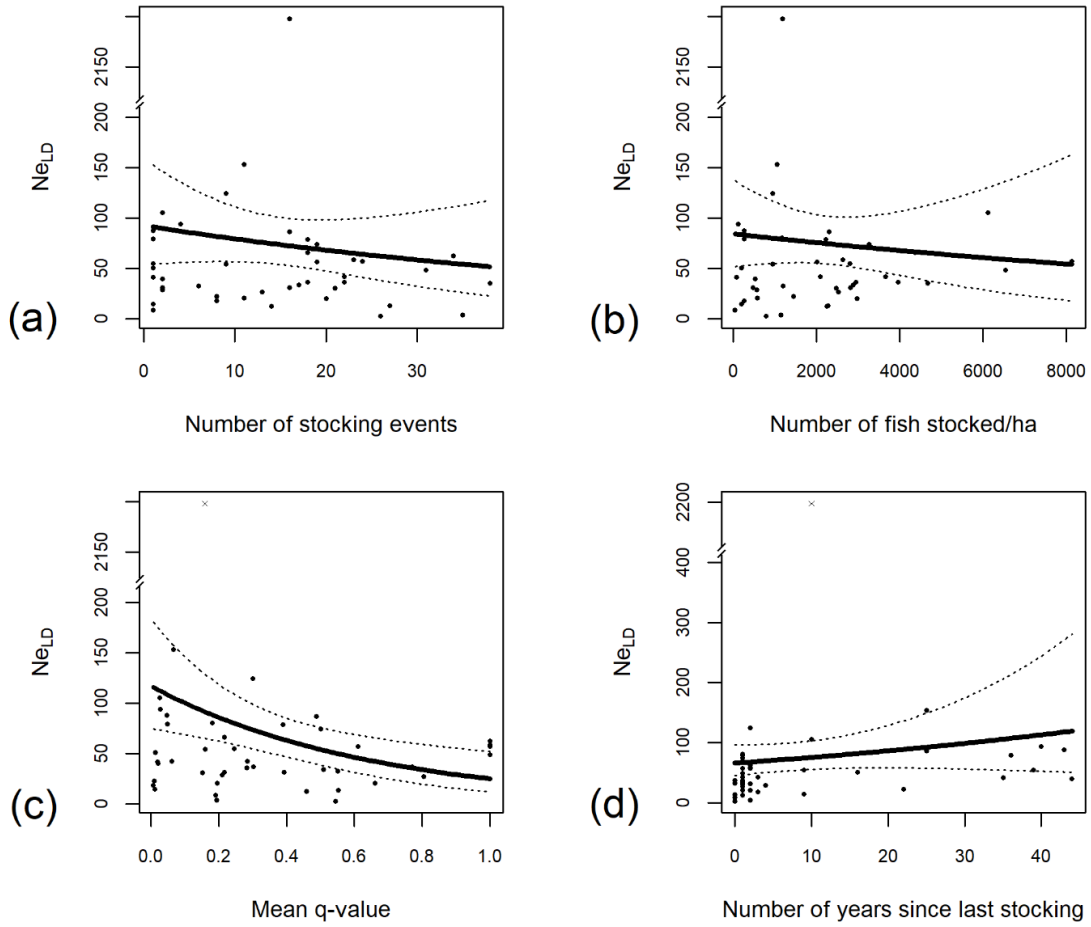

Figure 4.1 **Generalized linear models predictions of how effective population size (Ne_{LD}) varies with proxies of intensity of stocking including (a) number of stocking events, (b) number of fish stocked per hectare, (c) proportion of domestic genes in a lake and (d) number of years since last stocking event.** Explanatory variables have been unscaled for graphical representation. Only mean q-value has a significant effect on Ne here (c). When the lake 14-16_MIL (represented by a cross in c and d) is removed, the relationship with mean q-value becomes marginally non-significant and the relationship with the number of years since last stocking becomes significant. Full lines are the predictions of the models including the variables presented here and the wildlife reserve as fixed effects, dotted lines represent the 95% confidence intervals and black dots represent raw data. Note that the scale of Ne_{LD} was cut for illustrative purposes.

The removal of populations with a high LD did not change any of our results (Table S4.A7 to S4.A10). However, it should be noted that the only stocked population with an infinite estimate of Ne for the higher confidence interval (14-16_MIL) seemed to have a strong influence on two of our results. First, when this population was removed, the relationship between the proportion of domestic genetic background and Ne was weaker and only marginally non-significant (β (SE) = -0.16 (0.10), z = -1.64, p = 0.097, Table 4.3). Second, without this population, the number of years since last stocking was positively and significantly related to Ne (β (SE) = 0.23 (0.09), z = 2.50, p = 0.012, Table 4.3). No other result was affected by the removal of this population.

Discussion

In the present study, we provide empirical evidence that stocking decreases Ne in Brook Charr populations, but we also show that stocking intensity has little additional influence on the extent of Ne depletion, and that the long-term continuation of adverse effects of stocking on Ne is equivocal. We detail each of these results in specific sections below.

Effect of stocking on Ne

Our results indicate that stocked lakes have lower Ne than lakes that were never stocked, suggesting a negative effect of stocking. This depletion of Ne suggests that stocking hatchery-reared individuals could have increased variance in reproductive success in supplemented lakes since it is one of the main factor influencing Ne (Frankham 1995; Ardren and Kapuscinski 2003) and the usual explanation for Ne depletion after stocking (Ryman and Laikre 1991; Araki et al. 2007b). In our study system, stocked individuals are not issued from supportive breeding (i.e. they do not originate from the lakes in which they are stocked) and it is thus unlikely that the Ne depletion is attributed to the enhancement of the reproductive success of a portion of the

population in hatchery as described in Ryman and Laikre (1991). The observed effect of stocking on Ne here is more likely to be explained by a poor fitness of stocked fish that increases variance in reproductive success among breeding individuals as suggested by Araki et al. (2007b). Inadvertent selection during the process of domestication has been widely shown to lower reproductive success in domestic salmonids (Araki et al. 2008; Araki and Schmid 2010; Christie et al. 2012b) and stocking results in the cohabitation of wild and domestic individuals, which may have very different levels of reproductive success.

The deleterious effects of supplementation on Ne could also be explained by the stocking impacts on genetic characteristics of populations, for instance through its effect on heterozygosity (positive, Marie et al. 2010, or negative, Nock et al. 2011), or inbreeding rate (various effects, Duchesne and Bernatchez, 2002). Since these genetic features are closely linked to Ne (Frankham 1995), it is likely that stocked populations differ from unstocked populations in terms of Ne because of modifications of genetic structure due to the introduction of exogenous individuals.

Another possible explanation for our results would be that admixture between wild and domestic individuals artificially generates low Ne estimates in stocked lakes. Indeed, LD method of Ne estimation is based on the assumption that a strong LD is attributable to an important genetic drift and therefore to a small Ne (Hill 1981; Luikart et al. 2010). However, when a wild population is supplemented with domestic fish, it induces the coexistence of individuals with distinct genetic backgrounds in the same habitat, a situation that may artificially generate a high LD ("admixture linkage disequilibrium", Stephens et al. 1994; Waples 2006). Thus, the observed difference of Ne between stocked and unstocked populations may be due to a direct effect of admixture rather than to a deleterious effect of supplementation.

Effect of stocking intensity on Ne

Our results are equivocal regarding the influence of intensity of stocking on Ne of stocked populations. First, it seems that Ne is not affected by whether lakes are slightly or intensively supplemented, which suggests that, rather than a continuous relationship between stocking intensity and Ne, stocking *per se* could be the main driver of Ne depletion in our system. Indeed, we found no relationship between Ne and the number of stocking events or the number of fish stocked per hectare. This result is surprising considering that if the negative effect of stocking is attributable to an increase of variance in reproductive success due to the introduction of domestic individuals (Ryman and Laikre 1991; Araki et al. 2007b), Ne should be more strongly impacted in massively stocked lakes since a larger proportion of hatchery-reared individuals should contribute to reproduction (Christie et al. 2012a). A possible explanation for our results would be that the number of stocking events or the number of fish stocked per hectare are not representative of the reproductive contribution of domestic fish to the population. For instance, some lakes can be stocked numerous times with few individuals or with a high density of individuals only once, resulting in a limited domestic genetic contribution to the stocked population, and hence in a low impact on Ne. Moreover, the number of fish stocked in our study system is often representative of the development stage of stocked individuals, with early stages (e.g. fry) stocked in far higher numbers than older individuals (e.g. over one year old). Since mortality is strongly stage-dependent in fishes, with younger individuals experiencing higher mortality (Stringer et al. 1980; Valiant and Smith 1983), the number of fish stocked per hectare may not be a reliable proxy of the reproductive contribution of domestic fish to the population. Nevertheless, we detected a negative relationship between the proportion of domestic genetic background and Ne in stocked lakes. The proportion of domestic genetic background in the supplemented populations is a proxy of the persistence and/or reproduction of stocked individuals. It is linked to density of fish stocked, number of stocking events and number of years since last stocking (Létourneau et al. 2018, Table S4.A3), but it also quantifies the genetic contribution of hatchery fish, which is the actual driver of an effect of stocking on Ne. Thus, the negative relationship we found between the proportion of domestic genetic background and Ne

likely reflects the fact that a higher genetic contribution of domestic individuals has a stronger impact on Ne. It has also already been suggested that domestic introgression in wild populations could cause a decrease of Ne after stocking (Hansen et al. 2009). However, it should be noted that this relationship between Ne and the proportion of domestic genes was mainly driven by a population (14-16_MIL) having a very high Ne in our study. When this population was removed from the analysis, the relationship between the proportion of domestic background and Ne was only marginally non-significant. Thus, evidence of the impact of the proportion of domestic genetic background is still equivocal and overall, it seems that intensity of stocking has little effect on Ne depletion in our system.

The absence of a clear relationship between intensity of stocking and Ne, in our results, is somewhat surprising given that we detected a highly significantly negative impact of stocking *per se*. Several non-exclusive reasons could explain these results. First, if stocking decreases Ne, the variance in Ne of stocked lakes becomes too small to detect an effect of stocking intensity. Indeed, it has been shown in our study system that stocking results in genetic structure homogenization among supplemented lakes (Marie et al. 2010; Lamaze et al. 2012). Therefore, if stocked lakes become genetically similar because of supplementation, it is likely that their Ne also become close. This is supported by the fact that the variance of Ne for unstocked lakes (σ^2 = 9 667 474) is much larger than the variance of Ne for stocked lakes only (σ^2 = 106 013). Another possible explanation could be that stocked lakes are supplemented because they consist of poor environments, with less productive populations that have inherently low Ne. Indeed, it has been shown in the same system that diversity and prevalence of parasites were lower in stocked lakes, even though at the individual level no difference was found between domestic and wild individuals, suggesting that stocked lakes were low-quality environments (Gossieaux et al. 2018). Moreover, Brook Charr populations have been shown to display a wide range of Ne or Nb (the number of breeders in one reproductive cycle, a parameter closely linked to Ne) values depending on environmental characteristics and how they shape population dynamics (Wood et al. 2014; Bernos and Fraser 2016; Ruzzante et al. 2016). For instance, stocked lakes are supplemented to increase fishing rates and are thus subject to higher angling pressure. Yet,

it has been suggested that through the removal of a consequent portion of breeding adults, which reduces recruitment, fishing could reduce Ne (Kuparinen et al. 2016). Thus, lakes undergoing strong angling pressure are more likely to have depleted Ne. In this case, the difference of Ne between stocked and unstocked lakes would not be attributable to stocking but rather to their intrinsic characteristics.

The absence of relationship between lake area and Ne was also surprising, since genetic diversity is often predicted to be positively correlated to habitat size, suggesting that larger habitats should be associated with larger Ne (Frankham 1996; Hansen et al. 2009). However, Wood et al. (2014) showed that habitat size is less important in determining population size or Nb than habitat heterogeneity, which could partly explain our results, although this remains to be assessed further. It should be noted, however, that an analysis on a subsample of our populations (n = 28) showed no correlation between lake area and a proxy of Brook Charr population density (Table S4.A11), suggesting that lake area in our system may not be predictive of population size.

Effect of stocking interruption on Ne

Our results suggest that after cessation of stocking, Ne remains low, since the number of years since last stocking event was not related to Ne in our analyses. If the decrease of Ne is a consequence of stocking, this result indicates that this depletion could be permanent, or at least that Ne could remain low during decades after stocking has stopped. Another possible interpretation of this result is that the difference in Ne between stocked and unstocked lakes is due to environmental quality being a confounding factor in our analysis, with stocked lakes being poorer environments, as suggested in Gossieaux et al. (2018). In this case, there would be no expectation for Ne to increase after stocking stops since low Ne would be attributable to environmental differences rather than to an actual effect of stocking. To better separate the

environmental effects from the impacts of stocking on Ne, historical data could be used to compare Ne before and after stocking occurred. If such data are not available, environmental quality of the different environments could be accounted for to disentangle genetic effects linked to stocking from those intrinsic to the studied populations.

It should also be noted that the presence of one of our populations (14-16_MIL) with a high Ne seemed to conceal a significant positive relationship between the number of years since last stocking and Ne. When this population was removed, the relationship became significant, meaning that the more recent the last stocking event occurred in a lake, the lower the Ne. It has been shown that after the end of supplementation, domestic alleles can be purged from stocked populations (Valiquette et al. 2014; White et al. 2018), and notably in our study system (Létourneau et al. 2018). Although equivocal, since there is no significant effect of the proportion of domestic genes on Ne when 14-16_MIL is removed from the analysis, this result could suggest that the purge of domestic genes from stocked populations could explain the decline of the influence of stocking over time.

Estimating Ne in natural contexts

Estimators of Ne rely on several assumptions (e.g. no mutation, no selection, closed population, discrete generations) that are rarely all met in natural contexts. In our study, two assumptions were violated by the fact that first, Brook Charr have overlapping generations and second, that we are in a context of stocking and thus populations are not closed. In case of overlapping generations, Ne estimates obtained with the LD method lie between Nb (Waples et al. 2013, 2014) and the true Ne when multiple cohorts are sampled (Waples 2006; Luikart et al. 2010) as in our study. Moreover, the estimation becomes approximately equal to Ne when the sample includes as many cohorts as there are in a generation (Waples et al. 2014). For Brook Charr, generation time is 2 to 3 years at our latitudes (COSEWIC 2000; Kazyak et al. 2016; Ruzzante

et al. 2016) and, given the range of sizes we sampled in each population (Fig. A.1 and A.2), our Ne estimates should be close of the true Ne.

Since our populations are not closed, migration can bias upwardly or downwardly Ne estimates depending on the migration rate and genetic difference between the mixing populations (Waples and England 2011). When migration rate exceeds 5-10% of the population size, Ne estimates tend towards the metapopulation Ne (upward bias), while when the mixing populations are genetically distinct, it creates admixture LD (downward bias, Waples and England 2011). We already addressed the latter case previously in our discussion as a possible explanation for lower Ne of stocked lakes compared to unstocked lakes. When only considering stocked populations (i.e. our stocking intensity analyses), Ne of stocked lakes should at worst tend to Ne of the metapopulation that includes the lake and the hatchery populations, if migration rate is higher than 10%. In our system, lakes from Portneuf reserve are stocked with the Jacques-Cartier strain that has the highest Ne among hatcheries and which has a higher Ne than all but three lakes form the Portneuf Reserve. This suggest that for this reserve at least, the potential bias that could arise due to migration from hatcheries would not reduce our Ne estimates.

Finally, we followed the recommendations of Waples (2016) who advocates the use of more than one estimator to improve our ability to estimate Ne. We obtained similar estimates with both LD and sibship assignment methods (Table S4.A4), however, there was no relationship between these estimates and those obtained with the temporal method. This could be explained by the lower number of populations for which we could calculate Ne_{TM} (n = 12). This discrepancy could also be explained by the fact that the LD method estimates the inbreeding Ne (Ne_I) while the temporal method estimates the variance Ne (Ne_V, Luikart et al. 2010), two subcategories of Ne that can be differently affected by stocking (Wang and Ryman 2001).

Conclusions and perspectives

Even though our results show lower Ne in stocked populations, we cannot determine whether this is due to an actual detrimental effect of stocking or if stocked populations have lower Ne because of intrinsic populations features such as poorer environmental conditions. Nonetheless, the low Ne in stocked lakes we report suggests that stocking as a management measure should be assessed carefully since it may affect long-term persistence of populations. Indeed, populations of conservation concerns often display lower Ne and are thus less likely to persist because of genetic stochasticity (Palstra and Ruzzante 2008). Given the alteration of the genetic structure of our populations following stocking (e.g. Marie et al. 2010), a better understanding of the effects of stocking is important to avoid collapse of Ne in our populations, as it has been observed in other species (e.g. Tringali and Bert 1998), which could potentially lead to population extinction (Palstra and Ruzzante 2008).

At a larger scale, potential detrimental effects of stocking on Ne should be accounted for as supplementation often targets populations that are either vulnerable or exploited (Utter 2004; Naish et al. 2008; Laikre et al. 2010) and are thus more likely to be affected by a reduction of Ne. A possible solution to limit adverse effects of stocking on wild populations would be equalization of family size in hatcheries to restrain variance in reproductive success (Waples and Do 1994; Wang and Ryman 2001; Theodorou and Couvet 2004; Christie et al. 2012a). It should be noted that, with appropriate precautions, stocking can have little negative effects or even positive effects on Ne when contribution of spawners is balanced (e.g. Hedrick et al. 2000), or when a major increase in census size compensates the effects of the increase of variance in reproductive success (Wang and Ryman 2001).

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Compliance with Ethical Standards

All protocols and procedures employed were reviewed and approved by the Ministère des Forêts, de la Faune et des Parcs.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data availability

All data will be made available on Dryad.

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CHAPITRE 5
DISCUSSION ET CONCLUSION GÉNÉRALE

Discussion

Retour sur les résultats

La compréhension des conséquences génétiques et écologiques des ensemencements est cruciale pour en garantir l'efficacité sur le long terme, mais aussi pour la préservation des populations ensemencées. Des études précédentes ont permis de mieux caractériser les liens entre ensemencements et introgression génétique, ainsi que les facteurs qui modulent l'intensité de cette dernière. Ainsi, les causes de l'introgression génétique ont pu être éclaircies. Mes travaux de thèse s'inscrivent dans la continuité de ces études, afin de mieux comprendre quelles sont les conséquences d'une part des ensemencements, et d'autre part de l'introgression de gènes domestiques sur des paramètres populationnels mais aussi individuels. J'ai pour cela étudié des populations d'ombles de fontaine présentant des historiques d'ensemencements variés et donc des niveaux d'introgression diversifiés. Mes premières analyses ont été ciblées au niveau individuel et avaient pour but de comprendre comment l'introgression et l'origine génétique agissent sur le phénotype des individus (Chapitre 2). Mes résultats ont montré un effet significatif mais faible du bagage génétique sur la morphologie et la croissance. De plus, cet effet semble varier selon l'environnement dans lequel les individus se trouvent. Ces analyses ont également mis en évidence le fait que les poissons domestiques se nourrissent dans des niches trophiques plus benthiques et à des niveaux trophiques plus élevés que les poissons sauvages et hybrides ce qui pourrait expliquer une partie des différences phénotypiques observées. Cependant, il ressort globalement de ces analyses que le principal déterminant de la variation phénotypique est l'environnement. J'ai par la suite étudié la façon dont les relations

hôtes-parasites étaient influencées par les ensemencements et l'introgression (Chapitre 3). La première partie de ces analyses s'est concentrée au niveau individuel pour déterminer comment le bagage génétique pouvait influencer la capacité des poissons à se défendre contre les infections parasitaires. Aucun effet de l'origine génétique n'a été trouvé, suggérant que la domestication n'a pas altéré les capacités de défense contre le parasitisme. Dans un second temps, j'ai voulu déterminer si les niveaux d'introgression génétique des lacs pouvaient expliquer des variations de prévalence ou de diversité de la faune parasitaire. Mes résultats indiquent un effet significatif négatif de la proportion de gènes domestiques dans les populations sur ces paramètres. Cet effet était surprenant d'une part car aucun effet n'avait été détecté au niveau individuel, et d'autre part parce qu'il allait dans le sens opposé à celui de nos prédictions. En effet, il était attendu que les populations plus domestiques soient plus sensibles aux parasites. Or, les lacs ayant les plus grandes proportions de gènes domestiques présentaient des prévalences plus faibles ainsi qu'une moins grande diversité de parasites. Compte tenu de l'absence d'effet génétique au niveau individuel, les effets populationnels observés ici sont probablement expliqués par des facteurs confondants non mesurés. Nous avons vu dans le chapitre 2 que l'environnement semble avoir une importance capitale sur le phénotype et il est probable qu'il influence également très fortement les relations hôtes-parasites. Les lacs présentant de forts niveaux d'introgression ici auraient donc des prévalences plus faibles et une moins grande diversité de parasites non pas à cause de l'introgression mais parce que ces populations sont dans des environnements qui favorisent à la fois l'introgression génétique et une faune parasitaire moins diversifiée. Notamment, les lacs dans lesquels se retrouvent le plus de gènes domestiques sont probablement ensemencés plus fortement et sont donc potentiellement des environnements de moins bonne qualité dans lesquels les populations ne sont pas assez productives pour soutenir un effort de pêche. Ainsi, il pourrait être plus difficile pour les communautés de parasites de s'y établir en raison d'une forte mortalité des hôtes. Enfin, j'ai voulu mieux comprendre les effets des ensemencements et de leur intensité au niveau populationnel, plus spécifiquement sur les tailles effectives (Chapitre 4). Les résultats ont, dans un premier temps, montré que les populations n'ayant jamais été ensemencées ont des tailles effectives supérieures à celles ensemencées une fois ou plus. En utilisant plusieurs indicateurs de l'intensité des ensemencements, les analyses n'ont cependant mis en évidence que peu de

différence entre les tailles effectives des lacs légèrement et fortement ensemencés. Là encore, ces résultats indiquent que les lacs ensemencés diffèrent des autres mais que cette différence est difficilement attribuable aux ensemencements puisque leur intensité n'a que peu ou pas d'importance. De la même façon que dans le chapitre 3, une explication à ces résultats serait que certains lacs partagent des caractéristiques, probablement environnementales, qui font qu'ils ont à la fois des tailles effectives faibles et de plus grandes chances d'être ensemencés. Les prochaines sections seront consacrées à une discussion sur l'importance de l'environnement pour les variables utilisées dans mon travail, ainsi que sur de nouvelles perspectives pour approfondir notre compréhension de l'importance des conditions environnementales sur les conséquences des ensemencements, tout en soulignant certaines limites de mon étude.

Importance de l'environnement sur les variables étudiées

L'environnement au sens large (facteurs biotiques et abiotiques) est un facteur déterminant pour beaucoup des variables utilisées tout au long de mon doctorat. Il a par exemple été montré chez l'omble de fontaine que des facteurs environnementaux peuvent avoir une influence significative sur les niveaux d'introgression (Harbicht *et al.*, 2014), y compris dans notre système d'étude où l'introgression est plus importante dans les habitats de moins bonne qualité (Marie *et al.*, 2012, mais voir Létourneau *et al.*, 2018). Le contexte environnemental dans lequel se trouvent les individus ou dans lequel ils ont passé les premiers stades de leur vie est souvent un déterminant important du phénotype des salmonidés, notamment en termes de morphologie (Fleming *et al.*, 1994; Swain *et al.*, 1991; Zastavniouk *et al.*, 2017) et de croissance (Crespel *et al.*, 2012; Solberg *et al.*, 2013b; Yamamoto et Morita, 2002). L'environnement dans lequel se trouvent les individus peut également influencer leurs relations hôtes-parasites. Par exemple, bien que la température de l'eau ait un effet fort sur la présence de parasites dans certains habitats, elle peut interagir de façon complexe avec des facteurs biotiques spécifiques à certains milieux pour façonner la faune parasitaire et donc les niveaux d'infection des hôtes (Karvonen *et al.*, 2013; Strepparava *et al.*, 2018). D'un point de vue populationnel, les communautés de

parasites au sein des différentes populations d'hôtes sont également affectées par les caractéristiques des habitats tels que la profondeur (Bergeron *et al.*, 1997; Klimpel *et al.*, 2006). Enfin, les tailles effectives peuvent elles aussi dépendre du contexte environnemental dans lequel se trouvent les populations. Entre autres, la taille des habitats, leur degré de fragmentation ou encore l'intensité de la compétition sexuelle peuvent les moduler (Bernos et Fraser, 2016; Ruzzante *et al.*, 2016; Wood *et al.*, 2014).

Ainsi, toutes les variables réponses et certaines variables explicatives comme les niveaux d'introgression utilisées dans mon étude sont influencées de façon relativement importante par des facteurs environnementaux. J'ai pu intégrer quelques variables environnementales telles que la superficie des lacs, un indice de densité d'ombles de fontaine ainsi que celui d'un de leurs compétiteurs (le meunier noir, *Catostomus commersonii*) dans mes analyses sur le parasitisme (Chapitre 3). J'ai également utilisé la superficie des lacs dans mes analyses sur la taille effective (Chapitre 4). Cependant, je ne disposais d'aucune autre donnée environnementale utilisable pour l'ensemble des lacs de mon étude. L'hétérogénéité inter-populationnelle observée dans la majorité de mes résultats, quelle que soit la variable réponse, reflète très probablement une hétérogénéité environnementale entre les lacs étudiés.

Par ailleurs, l'hétérogénéité environnementale au sein même des lacs étudiés est inconnue dans mon étude. Il aurait par exemple été intéressant de caractériser les habitats à l'intérieur même des lacs afin de déterminer si, au sein d'une population, certains habitats sont plus favorables. De plus, si certains habitats intra-lacs sont sélectionnées préférentiellement par un groupe d'individus, la méthode de capture utilisée dans mon étude (filets maillants) pourrait avoir une influence sur certains résultats. En effet, les filets se posent depuis le bord vers le centre des lacs et il est donc possible que dans les lacs de grand diamètre, les milieux pélagiques soient sous-échantillonnés par rapport aux milieux littoraux. Des études ont également mis en évidence des biais possibles des filets maillants quant à la taille des individus capturés qui serait sélectionnée selon le diamètre du maillage, ce qui pourrait générer des conclusions erronées sur la distribution

des individus dans les lacs (e.g. Finstad & Berg 2004). Étant donné que mes résultats sur les isotopes stables (Chapitre 2) suggèrent une utilisation différentielle de l'habitat selon l'origine génétique, il pourrait être important de tenir compte des biais possible de cette méthode d'échantillonnage dans les études à venir.

La prise en compte de variables environnementales aussi bien biotiques (ex. présence et densité de compétiteurs, de prédateurs, pression de pêche) qu'abiotiques (ex. température, taux d'oxygène dissous, profondeur des plans d'eau, turbidité, pH) permettrait donc probablement une compréhension plus fine et plus profonde des paramètres étudiés dans mes travaux. De plus, comparer des populations sur des critères phénotypiques chez une espèce aussi variable d'une population à l'autre que l'omble de fontaine (ex. Kazyak *et al.*, 2015; Zastavniouk *et al.*, 2017) est une tâche compliquée et comprendre le rôle de l'environnement dans cette variation pourra potentiellement permettre de mieux en tenir compte lors de futures études.

Enfin, dans un contexte de changements environnementaux à large échelle, comme c'est le cas aujourd'hui avec les impacts des activités humaines et les changements climatiques, déterminer l'ampleur de l'importance des facteurs environnementaux sur les individus et les populations d'espèces d'intérêt commercial ou à risque d'extinction pourrait permettre d'améliorer l'efficacité des programmes de conservation ou de gestion mis en place.

Pistes de recherche futures

Une limite importante dans l'interprétation de mes résultats et commune à mes trois chapitres est le fait que l'influence de l'environnement semble forte mais que je n'ai pas pu la quantifier. À l'avenir, il serait donc important de collecter des données environnementales lors des phases de terrain en même temps que des échantillons génétiques. Par ailleurs, afin d'identifier quels

sont les facteurs environnementaux qui affectent les variables étudiées ici, des expériences en milieu contrôlé pourraient être réalisées. En effet, un dispositif permettant d'ajuster artificiellement les variables environnementales d'intérêt telles que la température, le pH ou encore la densité de conspécifiques tout en gardant les autres variables identiques entre les groupes comparés permettrait d'isoler les variables affectant les traits étudiés et d'en comprendre l'influence. Par exemple, des expériences en jardin commun, bien que coûteuses et parfois difficiles à mettre en place, sont un excellent moyen d'étudier les phénomènes d'adaptations locales (Kawecki et Ebert, 2004). Ce type de protocole expérimental pourrait également permettre de tester les effets de l'origine génétique directement sur les traits étudiés (ex. Skaala *et al.*, 2019), mais aussi les interactions possibles entre le génotype et l'environnement (ex. Crespel *et al.*, 2013b; Solberg *et al.*, 2013b). En effet, bien que les effets génétiques soient dépassés par les effets environnementaux dans mon étude, ils n'en restent pas moins significatifs pour un certain nombre de variables (ex. prévalence, diversité de parasites, taille effective), parfois en interaction avec l'environnement (ex. morphologie, croissance, taille à l'âge). Contrôler pour les facteurs environnementaux permettrait alors de quantifier précisément leur importance.

De nombreux autres traits individuels que ceux étudiés ici sont affectés par le processus de domestication et sont donc susceptibles d'être modifiés à la suite d'épisodes d'introgression génétique. Il aurait par exemple été intéressant de mesurer la réponse au stress et de la comparer entre les individus selon leur origine génétique. En effet, la sélection artificielle en pisciculture cible souvent ce trait afin d'obtenir des poissons plus résistants au stress (Solberg *et al.*, 2013a) et des différences entre individus domestiques et sauvages ont déjà été rapportées (Crespel *et al.*, 2011; Solberg *et al.*, 2012; Woodward et Strange, 1987). Des contraintes techniques et logistiques ont cependant rendu la collecte de ce type de données impossible à mettre en place dans notre étude. Ce trait pourrait néanmoins être étudié dans un environnement contrôlé, tel que suggéré plus haut. De plus, il semble que le stress soit fortement et négativement lié à la croissance (Solberg *et al.*, 2013a; Vijayan et Leatherland, 1988), un trait pris en compte dans

mon chapitre 2. Il a d'ailleurs été suggéré que la croissance et la condition corporelle peuvent être considérées comme des indicateurs indirects des niveaux de stress (Barton, 2002).

Ultimement, l'objectif de cette étude était de déterminer si les individus introgressés avaient une aptitude phénotypique réduite, et si cela pouvait avoir un impact sur la persistance des populations ensemencées. Afin de répondre au mieux à cette question, l'évaluation directe de l'aptitude phénotypique au travers de données sur la survie et le succès reproducteur aurait été idéale. La collecte de ces données n'a pas été possible, c'est pourquoi des indicateurs plus indirects de l'aptitude phénotypique ont été utilisés. Des études précédentes ont déjà mis en évidence des effets négatifs de la domestication sur la survie (Fraser, 1981; McGinnity *et al.*, 1997, 2003; Reisenbichler et McIntyre, 1977; Reisenbichler et Rubin, 1999; Webster et Flick, 1981) et le succès reproducteur (Araki *et al.*, 2007a, 2007b; Christie *et al.*, 2014; Jonsson et Jonsson, 2006; Leonard *et al.*, 2013; Williamson *et al.*, 2010). L'introgression génétique entre individus sauvages et domestiques semble également altérer la survie (Fraser, 1981; McGinnity *et al.*, 1997, 2003; Reisenbichler et Rubin, 1999; Solberg *et al.*, 2013b; Webster et Flick, 1981) et le succès reproducteur (Araki *et al.*, 2007a, 2008, 2009, Christie *et al.*, 2012a, 2014; Lachance et Magnan, 1990; Muhlfeld *et al.*, 2009; Whiteley *et al.*, 2009; Williamson *et al.*, 2010, mais voir Berejikian *et al.*, 2009) bien que cet effet ne soit pas systématique (Araki et Schmid, 2010). Cependant, les mécanismes sous-jacents à cette réduction de l'aptitude phénotypique ne sont pas toujours bien compris. Il a par exemple été suggéré que la diminution de survie liée à la domestication pourrait être liée à une diminution des comportements anti-prédateurs (Tymchuk *et al.*, 2007) ou de la performance de nage (Reinbold *et al.*, 2009) suite à l'élevage. Il reste donc intéressant d'étudier des traits liés à l'aptitude afin de mieux comprendre quelles sont les causes sous-jacentes potentielles de cette diminution de performance.

Un autre volet qu'il aurait été intéressant d'explorer est celui de l'impact de la domestication sur la vulnérabilité à la pêche. En effet, la domestication implique des modifications comportementales (Biro et Post, 2008; Price, 1999) telles qu'une prise de risques accrue (Biro

et Sampson, 2015; Biro *et al.*, 2004), une augmentation de l'activité (Biro *et al.*, 2004; Cooke *et al.*, 2007), ou encore de l'exploration (Härkönen *et al.*, 2014). Un certain nombre de ces changements comportementaux sont associés à la croissance plus importante des individus domestiques (Biro et Post, 2008; Biro et Sampson, 2015; Biro *et al.*, 2004; Huntingford, 2004). Or, certains traits comportementaux peuvent directement permettre de prédire la vulnérabilité à la pêche des individus, indépendamment de leur taille (Härkönen *et al.*, 2014) et il est donc possible que les individus domestiques soient plus susceptibles d'être pêchés (Flick et Webster, 1962). La collecte de données associées à cette question était prévue lors des phases d'échantillonnage de mon projet mais les effectifs récoltés étaient trop faibles pour pouvoir exploiter ces échantillons. Cela reste cependant réalisable si d'autres campagnes d'échantillonnages sont mises en place et serait une voie intéressante à explorer tant d'un point de vue écologique que pour les gestionnaires.

Enfin, il serait intéressant de poursuivre l'échantillonnage de ces populations dans le futur. Les niveaux d'introgression génétique semblent diminuer lorsque les ensemencements sont interrompus, laissant la possibilité aux populations ensemencées de redevenir totalement « sauvages » (Létourneau *et al.*, 2018). Il serait intéressant de comparer alors l'occupation des niches trophiques benthiques, présentement préférentiellement occupées par des individus domestiques, afin de voir si les poissons sauvages retournent se nourrir dans ces niches ou si ces-dernières restent vacantes après la disparition des individus domestiques. De plus, poursuivre l'échantillonnage de ces populations pourrait permettre de surveiller de possibles changements temporels de phénotypes, de communautés parasitaires ou de tailles effectives, ce qui permettrait de mieux comprendre comment ces variables varient non pas seulement selon l'environnement dans lequel se trouvent les individus, mais également selon la variabilité environnementale temporelle.

Conclusion générale

À la suite de travaux précédents ayant démontré que les ensemencements causent l'introgression de gènes domestiques dans notre système d'étude (Marie et al. 2010, 1012), ma thèse avait pour but de comprendre quelles sont les conséquences de ces ensemencements et de l'introgression génétique sur des facteurs aussi bien individuels que populationnels dans des populations d'ombles de fontaine. Au travers des trois chapitres qui la composent, j'ai pu montrer que les ensemencements et l'hybridation entre individus domestiques et sauvages ont des effets modérés sur le phénotype, les relations hôtes-parasites et la taille effective des populations étudiées. J'ai en revanche montré une variation inter-populationnelle importante pour ces variables, bien que probablement non liée à la supplémentation des lacs avec des poissons de pisciculture. En termes de gestion des populations ensemencées, mes travaux ne suggèrent donc pas que les ensemencements soient néfastes en termes de pollution génétique comme cela a pu être suggéré dans la littérature. Cela semble également supporté par le fait que, dans notre système, les niveaux d'introgression diminuent lorsque les ensemencements cessent et que les lacs retrouvent des profils génétiques sauvages (Létourneau et al. 2018). Mes résultats permettent en revanche de mettre en lumière l'importance cruciale de l'environnement et de sa prise en compte lors d'études qui comparent des populations différentes. Ces conclusions pourraient être le point de départ à de nouveau travaux s'intéressant plus aux facteurs environnementaux qui façonnent les variables que j'ai étudiées ici. Il serait par exemple important de déterminer dans quelle mesure la pression de pêche pourrait faire partie de ces variables environnementales étant donné que les ensemencements sont pratiqués dans le but de maintenir des succès de pêche élevés et que la pression de pêche est une variable qui peut être contrôlée par les gestionnaires. Plus globalement, dans un contexte de changements environnementaux globaux, identifier les variables environnementales les plus importantes pour le phénotype, les relations hôtes-parasites et la taille effective pourra permettre une meilleure gestion des populations visées par des programmes de conservation ou de gestion.

ANNEXES

Annexes Chapitre 2

Appendix A: Supplementary information on methods and results.

Table S2.A1 List of lakes used for phenotypic analyses with respective effectives for analyses using morphometrics and growth data.

Reserve	Lake	Abbreviation	Total n sampled	n for morphometric analyses	n for growth analyses	Proportion of domestic genes (mean q-value) ± standard deviation	He	Ho
PN	Amanites	AMA	71	59	61	20% ± 34	0.58	0.51
PN	Caribou	CAR	32	23	25	20% ± 26	0.64	0.62
PN	Méthot	MET	50	45	45	28% ± 40	0.74	0.70
STM	Bec-Scie	BEC	41	32	38	66% ± 43	0.69	0.73
STM	Clairval	CLAI	40	32	33	15% ± 26	0.64	0.66
STM	Ecarté	ECAR	41	37	39	22% ± 28	0.70	0.72
STM	Est	EST	40	36	36	46% ± 47	0.68	0.69
STM	Milord	MIL	44	39	40	16% ± 27	0.71	0.70
STM	Perdu	PER	43	31	39	49% ± 34	0.64	0.65
STM	Pin	PIN	64	57	62	81% ± 36	0.72	0.78
STM	Plongeon-Huard	PLON	40	31	33	22% ± 23	0.68	0.76
STM	Soucis	SOU	41	35	36	25% ± 34	0.69	0.66

STM = Saint-Maurice, MAS = Mastigouche, PN = Portneuf, n = number of sampled individuals; He = expected heterozygosity, Ho = observed heterozygosity. Proportion of domestic genes = mean q-value of a population x 100.

Table S2.A2 List of lakes used for stable isotopes analyses with their effectives and values of introgression.

Lake	Genetic status	n with a 0.2-0.8 threshold	Proportion of domestic genes (mean q-value) ± standard deviation with a 0.2-0.8 threshold	Mean δ13C ± 95% CI with a 0.2-0.8 threshold	Mean δ15N ± 95% CI with a 0.2-0.8 threshold	n with a 0.1-0.9 threshold	Proportion of domestic genes (mean q-value) ± standard deviation with a 0.1-0.9 threshold	Mean δ13C ± 95% CI with a 0.1-0.9 threshold	Mean δ15N ± 95% CI with a 0.1-0.9 threshold
AMA	D	49	97%+-5	-26.7 [-28.4 - -25.1]	9.3 [9.1 - 9.5]	44	99%+-2	-26 [-27.7 - -24.4]	9.4 [9.2 - 9.6]
AMA	H	20	51%+-16	-32.1 [-33.3 - -30.7]	8.7 [8.4 - 8.9]	26	56%+-21	-32.4 [-33.3 - -31.2]	8.6 [8.3 - 8.8]
AMA	W	83	1%+-2	-33.1 [-33.4 - -32.8]	8.5 [8.4 - 8.6]	82	1%+-1	-33.1 [-33.4 - -32.8]	8.5 [8.4 - 8.6]
BEL	D	27	99%+-3	-25.4 [-27.5 - -23.4]	9.1 [8.8 - 9.4]	26	99%+-0	-25.6 [-27.8 - -23.6]	9.1 [8.8 - 9.4]
BEL	H	2	43%+-10	-32 [-32.7 - -31.3]	8.4 [8.2 - 8.7]	4	47%+-29	-26.1 [-32 - -20.1]	9.2 [8.4 - 10]
BEL	W	26	2%+-4	-30.5 [-31.6 - -29.1]	8.1 [7.7 - 8.4]	25	1%+-2	-30.9 [-31.7 - -29.8]	8 [7.7 - 8.3]
MER	D	27	99%+-2	-30.2 [-30.6 - -29.7]	8.7 [8.5 - 9]	27	99%+-2	-30.2 [-30.6 - -29.7]	8.7 [8.5 - 9]
MER	H	8	48%+-18	-31.8 [-33.2 - -30.4]	8.2 [7.5 - 8.7]	12	38%+-20	-31.7 [-32.7 - -30.6]	8.2 [7.8 - 8.6]
MER	W	47	4%+-5	-31.8 [-32.2 - -31.3]	8.7 [8.5 - 8.9]	43	2%+-2	-31.8 [-32.3 - -31.3]	8.7 [8.5 - 9]
MET	D	32	95%+-4	-25 [-26.7 - -23.4]	10.2 [10 - 10.4]	29	96%+-3	-25 [-26.7 - -23.4]	10.2 [10 - 10.5]
MET	H	21	47%+-18	-31.5 [-32.1 - -30.8]	9.1 [8.6 - 9.6]	35	40%+-24	-31.3 [-32.3 - -30.2]	9.2 [8.8 - 9.6]
MET	W	96	4%+-5	-32.2 [-32.6 - -31.7]	8.9 [8.6 - 9.1]	85	3%+-2	-32.1 [-32.6 - -31.7]	8.8 [8.6 - 9.1]

AMA = Amanites, BEL = Belles de Jour, MER = Mercure, MET = Méthot. AMA, BEL and MET are in the Portneuf reserve, MER is in the Mastigouche reserve. D = Domestic; H = hybrid; W = wild. Proportion of domestic genes = mean q-value of a population x 100.

Table S2.A3 Results of the Procrustes ANOVA using all lakes.

Variables tested	F	Z	Pr(>F)
Lake:q	1.36	3.69	<0.001
Cohort:q	0.96	1.78	0.04
Lake:cohort	1.04	3.74	<0.001
Fulton index	9.52	5.12	<0.001
Total length	3.93	3.57	<0.001
R^2	*36.7%*		

Table S2.A4 Results of the Tukey post-hoc tests of the effects of genetic origin on $\delta^{13}C$ and $\delta^{15}N$ ratios.

Lake	Genetic status	$\delta^{13}C$			$\delta^{15}N$		
		lwr	upr	p	lwr	upr	p
AMA	H-D	**-8.38**	**-4.38**	**<0.001**	**-1.14**	**-0.46**	**<0.001**
AMA	W-D	**-8.65**	**-5.63**	**<0.001**	**-1.16**	**-0.65**	**<0.001**
AMA	W-H	-2.58	1.06	0.70	-0.41	0.21	0.88
BEL	H-D	-6.32	5.48	0.98	-0.93	1.20	1.00
BEL	W-D	**-8.34**	**-2.19**	**<0.001**	**-1.63**	**-0.51**	**<0.001**
BEL	W-H	-10.76	1.07	0.20	-2.27	-0.14	0.04
MER	H-D	-2.81	-0.23	**0.03**	-1.12	0.14	0.24
MER	W-D	**-2.56**	**-0.74**	**<0.001**	-0.44	0.46	1.00
MER	W-H	-1.34	1.08	0.98	-0.10	1.09	0.21
MET	H-D	**-8.16**	**-4.54**	**<0.001**	**-1.77**	**-0.35**	**<0.001**
MET	W-D	**-8.71**	**-5.60**	**<0.001**	**-1.99**	**-0.77**	**<0.001**
MET	W-H	-2.26	0.64	0.52	-0.89	0.24	0.49

Genetic status (D = domestic; H = hybrid; W = wild) were determined with the 0.1-0.9 threshold of q-values ($q < 0.1$ = W; $0.1 < q < 0.9$ = H; $0.9 < q$ = D). Intervals are based on the Studentized range statistic with a 95% confidence level and are reported in columns "lwr" for the lower interval and "upr" for the upper interval. Values of p are presented here after the application of the False Discovery Rate (FDR) correction. Significant differences between groups ($p < 0.05$, intervals do not overlap 0) are in bold. Names of the lakes : AMA = Amanites; BEL = Belles de Jour; MER = Mercure; MET = Methot

Table S2.A5 Results of ANOVA on $\delta^{13}C$ data to determine if (a) genetic status and length (defined as length in cm between landmarks 2 and 11, see Fig. S2.A1) influence trophic niche for all fish, and if (b) length influence trophic niche in domestic fish only.

	Lake	Variables	n	Mean Sq	F-value	p
(a)	AMA	Genetic status	147	586.60	44.43	**<0.001**
		Length		17.00	1.29	0.26
	BEL	Genetic status	54	193.95	9.20	**<0.001**
		Length		2.44	0.12	0.73
	MER	Genetic status	74	21.27	11.35	**<0.001**
		Length		39.24	20.94	**<0.001**
	MET	Genetic status	145	651.10	81.13	**<0.001**
		Length		39.30	4.89	**0.03**
(b)	AMA	Length	47	1.28	0.04	0.85
	BEL	Length	26	8.41	0.26	0.61
	MER	Length	25	13.69	14.78	**<0.001**
	MET	Length	31	72.55	3.46	0.07

Genetic status (domestic, hybrid, wild) were determined with the 0.1-0.9 threshold of q-values ($q < 0.1$ = wild; $0.1 < q < 0.9$ = hybrid; $0.9 < q$ = domestic). Name of lakes: AMA = Amanites; BEL = Belles de Jour; MER = Mercure; MET = Methot

Figure S2.A1 Placement of the landmarks for morphometric analysis.

1 = extremity of the mandible; 2 = most anterior part of the body; 3 = anterior extremity of the eye; 4 = posterior extremity of the eye; 5 = point directly above the upper point of operculum; 6 = anterior basis of dorsal fin; 7 = posterior basis of dorsal fin; 8 = anterior basis of adipose fin; 9 = point directly above the thinnest part of caudal peduncle; 10 = dorsal junction point between body and caudal fin; 11 = most posterior point of the body excluding caudal fin; 12 = ventral junction point between body and caudal fin; 13 = point directly below the thinnest part of caudal peduncle; 14 = posterior basis of anal fin; 15 = anterior basis of anal fin; 16 = implantation of pelvic fin; 17 = implantation of pectoral fin; 18 = most posterior point of operculum.

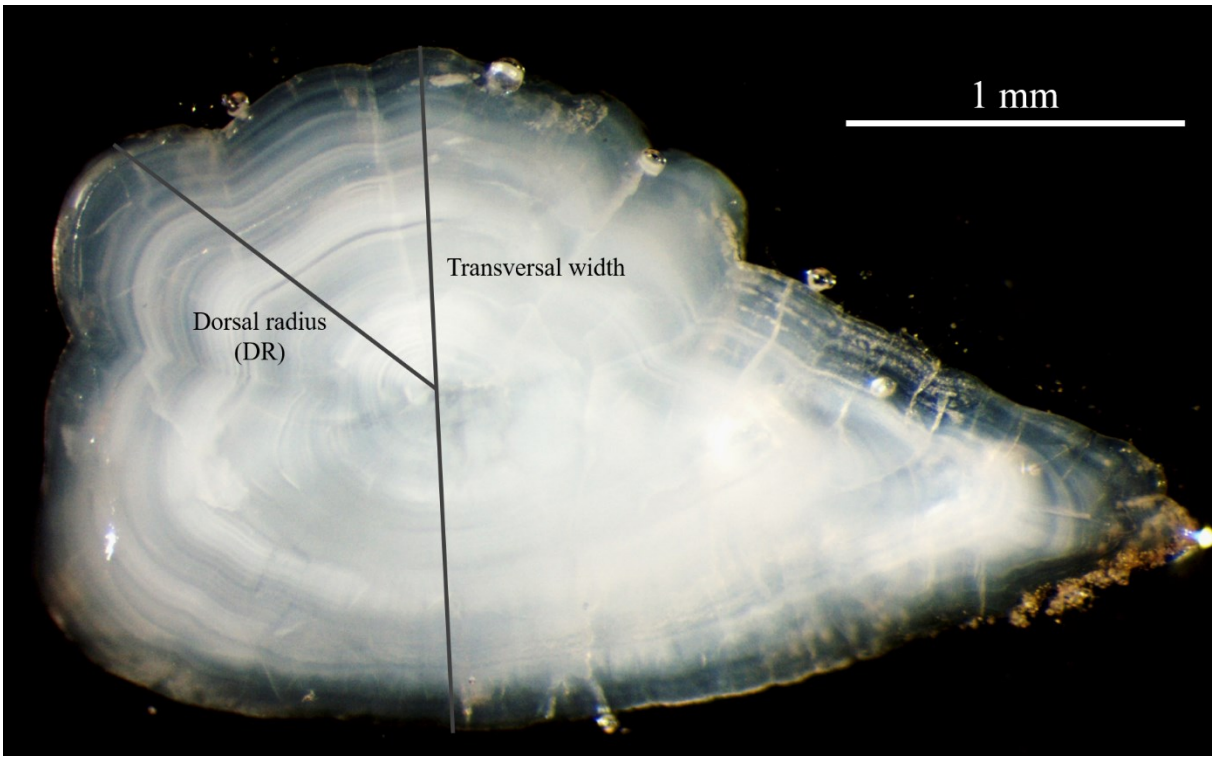

Figure S2.A2 Sagittal cut of an otolith.

Dorsal radius and transversal width are indicated here and measured for all otoliths. Image is zoomed 40 times**.**

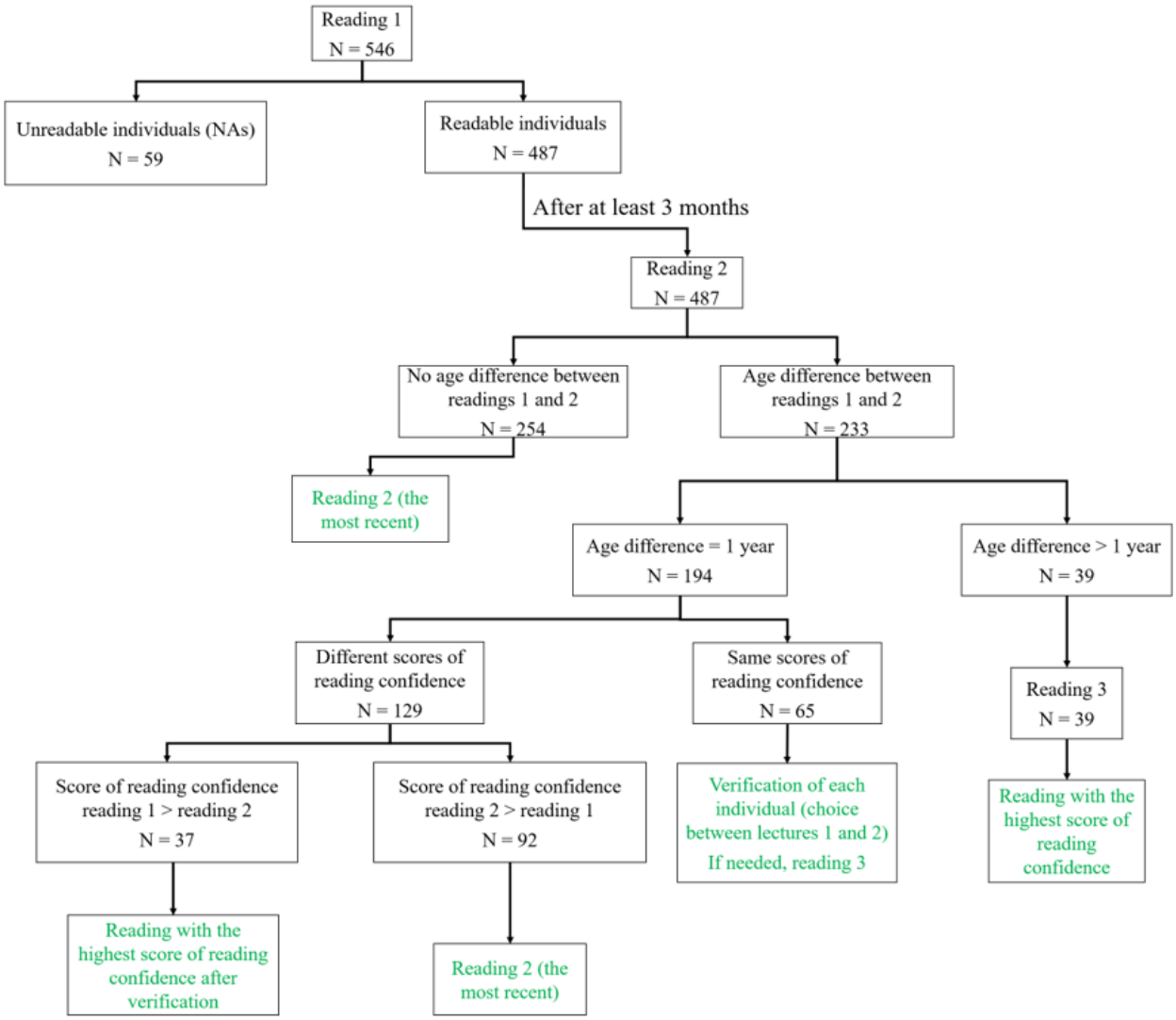

Figure S2.A3 Decision tree of otolith reading.

Green cases represent the readings kept for analyses. Effectives are specified in each case.

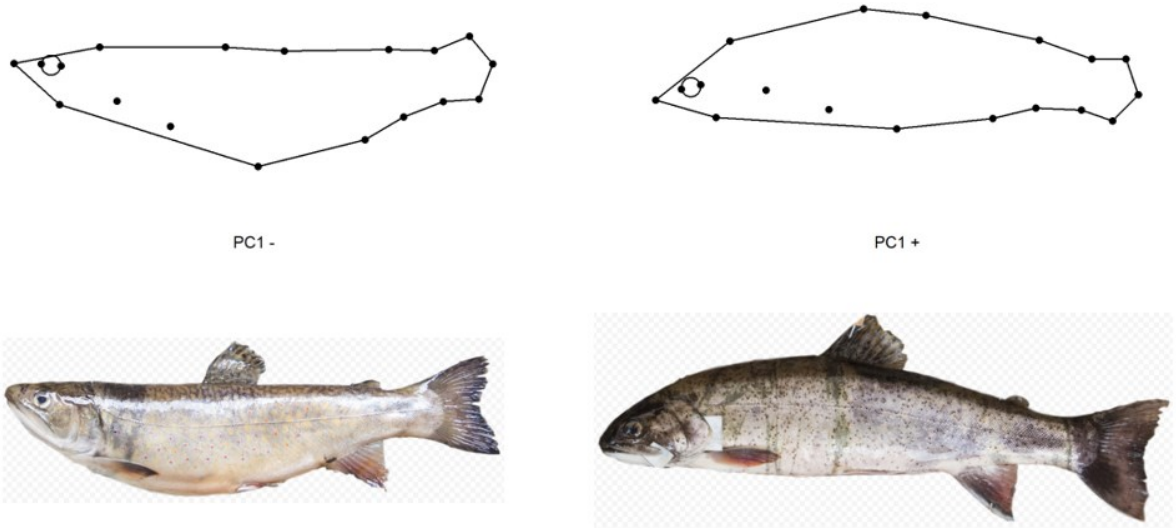

Figure S2.A4 Illustration of the extremes forms of PC1.

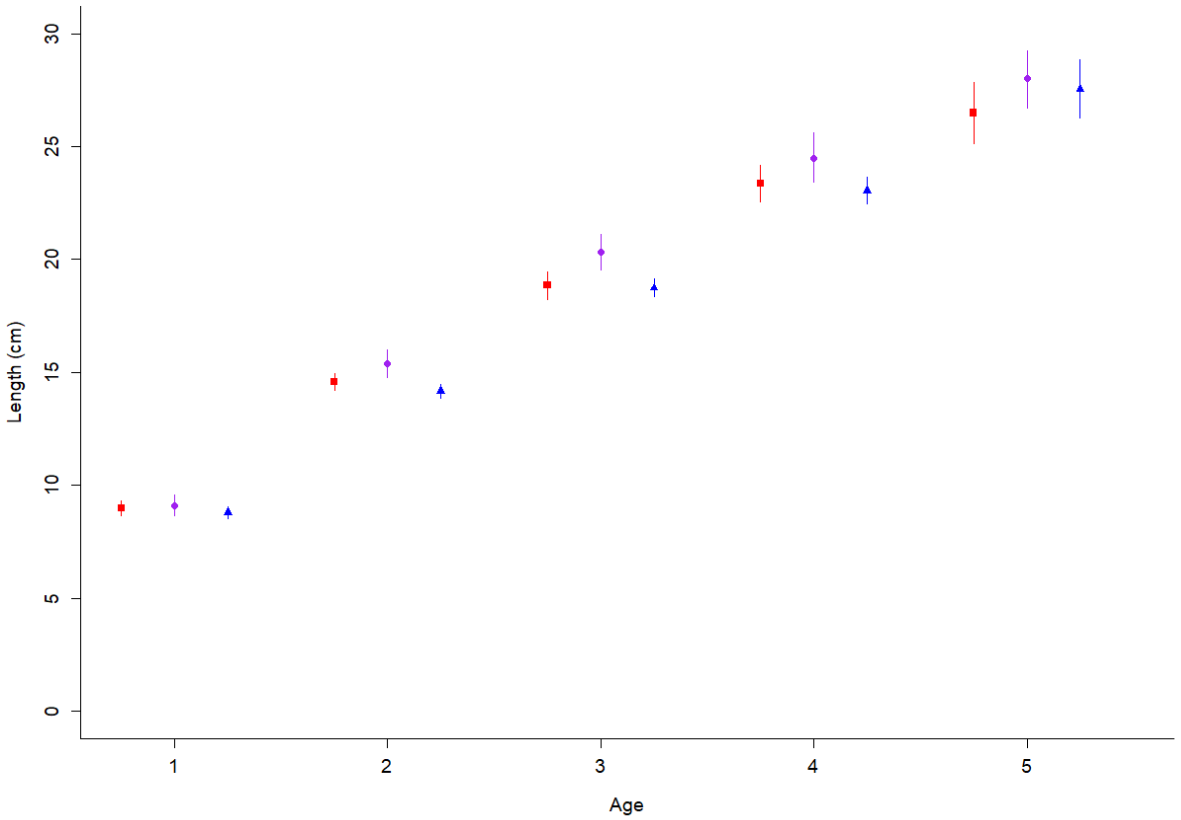

Figure S2.A5 Mean back-calculated length (cm) at each age with 95% confidence intervals.

Red squares, purple dots and blue triangles respectively represent domestic, hybrid and wild individuals.

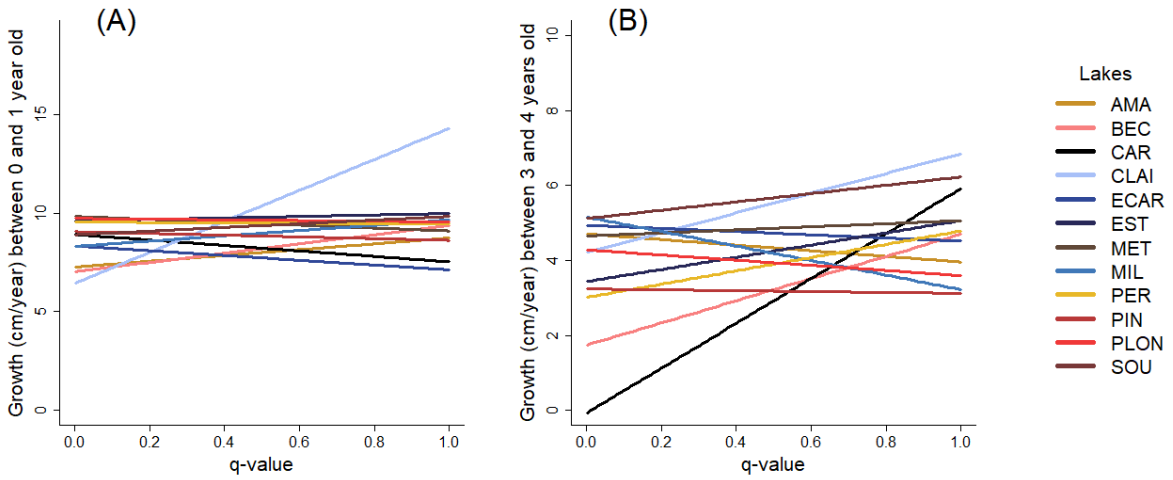

Figure S2.A6 Graphs of significant interactions between q-value and lake between (A) 0 and 1 year old and (B) 3 and 4 years old on growth (cm/year).

Complete names of lakes can be found in Table S2.A1.

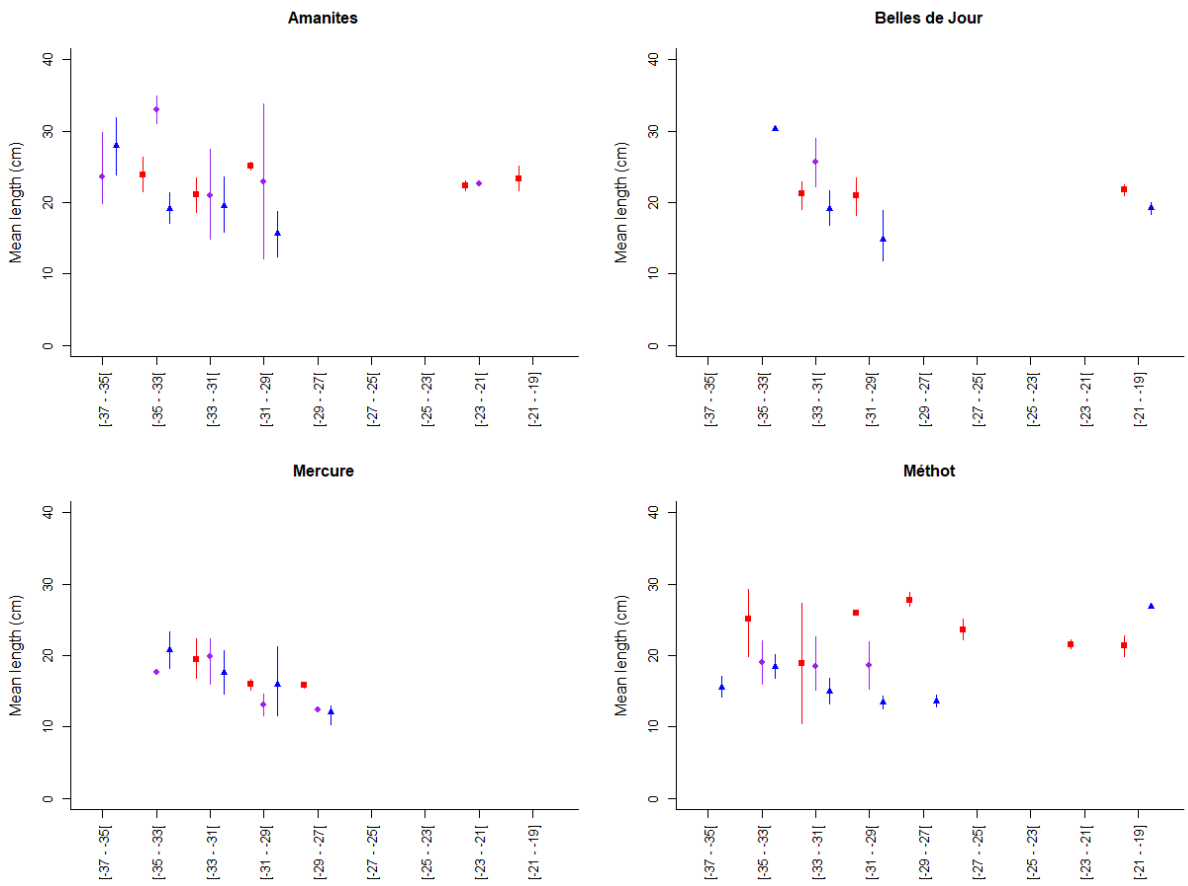

Figure S2.A7 Mean length (cm) with 95% confidence intervals for each genetic group along the $\delta^{13}C$ gradient for each lake.

Red squares, purple dots and blue triangles respectively represent domestic, hybrid and wild individuals.

Appendix B: Additional analyses results.

Results of morphometric and growth analyses with lakes from Saint-Maurice reserve only, with and without sex.

Table S2.B1 Results of the Procrustes ANOVA including only lakes from Saint-Maurice reserve.

Variables tested	F	Z	Pr(>F)
Lake:q	1.44	3.24	<0.001
Cohort:q	1.20	2.20	0.02
Lake:cohort	1.05	2.88	<0.01
Fulton index	6.99	4.42	<0.001
Total length	4.25	3.53	<0.001
R^2	*34.4%*		

Table S2.B2 F-values from backward stepwise selection of linear models on morphometric data (relative warp analysis, n = 330). Only lakes from Saint-Maurice reserve are in this analysis.

	n	Lake:q-value	Cohort:q-value	Lake:Cohort	Lake	Cohort	q-value	Fulton index	Total length	Adjusted R^2	Proportion of variance explained
PC1	330	0.94	0.98	0.94	**4.90**	1.03	0.24	1.58	1.24	*8.7%*	*40.4%*
PC2	330	**2.83**	**2.59**	**1.49**	**inter**	**inter**	**inter**	**46.29 (0.005)**	**14.01 (0.001)**	*42.9%*	*11.6%*
PC3	330	0.92	1.09	1.03	**3.63**	0.83	0.84	**22.31 (-0.003)**	0.17	*29.4%*	*8.2%*
PC4	330	**0.00**	0.58	0.11	**inter**	1.08	**inter**	0.07	0.11	*26.0%*	*7.4%*

Significant variables ($p < 0.05$) are in bold. Estimates are provided for significant continuous variables that are not in an interaction. Removal of variables that are in an interaction was not tested, thus we provide no value in these cases and indicate them with the term "inter".

Table S2.B3 Results of the Procrustes ANOVA including only lakes from Saint-Maurice reserve and sex (males, females or indeterminate) is included as an explanatory variable.

Variables tested	F	Z	Pr(>F)
Lake:q	1.36	3.03	<0.01
Cohort:q	1.09	1.92	0.03
Lake:cohort	1.02	2.73	<0.01
Fulton index	7.23	4.48	<0.001
Total length	4.18	3.52	<0.001
Sex	1.85	2.59	0.01
R^2	*35.3%*		

Table S2.B4 F-values from backward stepwise selection of linear models on morphometric data (relative warp analysis, n = 330). Only lakes from Saint-Maurice reserve are in this analysis and sex (males, females or indeterminate) is included as an explanatory variable.

	n	Lake:q-value	Cohort:q-value	Lake:Cohort	Lake	Cohort	q-value	Fulton index	Total length	Sex	Adjusted R^2	Proportion of variance explained
PC1	330	1.00	1.00	0.89	**4.90**	1.03	1.03	1.03	1.03	1.32	*10.9%*	*40.4%*
PC2	330	**2.51**	**2.31**	1.29	**inter**	**inter**	**inter**	**64.675 (0.006)**	**10.957 (0.0007)**	**7.47**	*31.6%*	*11.6%*
PC3	330	1.29	0.90	1.12	**3.26**	1.01	0.89	**20.328 (-0.003)**	0.00	**3.55**	*30.5%*	*8.2%*
PC4	330	**3.08**	1.05	1.36	**inter**	0.97	**inter**	0.00	0.07	**7.07**	*28.8%*	*7.4%*

Significant variables ($p < 0.05$) are in bold. Estimates are provided for significant continuous variables that are not in an interaction. Removal of variables that are in an interaction was not tested, thus we provide no value in these cases and indicate them with the term "inter".

Table S2.B5 F-values from backward stepwise selection of linear models on growth and size at each age ("YO" = years old). Only lakes from Saint-Maurice reserve are in this analysis.

	n	Lake:q-value	Cohort:q-value	Lake:Cohort	Lake	Cohort	q-value	Fulton index	Otolith reading confidence	Adjusted R^2
Length 1 YO	356	**2.18**	0.43	**2.02**	**inter**	**inter**	**inter**	1.17	0.45	*27.0%*
Length 2 YO	339	1.90	0.81	**2.08**	**inter**	**inter**	**7.16 (1.14)**	0.00	1.59	*21.8%*
Length 3 YO	231	1.69	0.21	**1.94**	**inter**	**inter**	**13.86 (2.12)**	0.36	3.75	*25.6%*
Length 4 YO	142	**3.56**	1.65	**1.88**	**inter**	**inter**	**inter**	**3.97 (-0.54)**	0.17	*36.1%*
Growth 0-1 YO	356	**2.18**	0.43	**2.02**	**inter**	**inter**	**inter**	1.17	0.45	*27.0%*
Growth 1-2 YO	339	0.42	0.59	**2.57**	**inter**	**inter**	1.78	1.09	**6.44 (0.27)**	*17.8%*
Growth 2-3 YO	231	0.51	0.67	1.46	**2.71**	0.19	1.97	0.37	**5.42 (0.22)**	*6.8%*
Growth 3-4 YO	142	1.87	1.10	1.66	**3.32**	1.97	1.58	0.43	0.04	*11.6%*

Significant variables ($p < 0.05$) are in bold. Estimates are provided for significant continuous variables that are not in an interaction. Removal of variables that are in an interaction was not tested, thus we provide no value in these cases and indicate them with the term "inter".

Table S2.B6 F-values from backward stepwise selection of linear models on growth and size at each age ("YO" = years old). Significant variables ($p < 0.05$) are in bold. Only lakes from Saint-Maurice reserve are in this analysis and sex (males, females or indeterminate) is included as an explanatory variable.

	n	Lake:q-value	Cohort:q-value	Lake:Cohort	Lake	Cohort	q-value	Fulton index	Otolith reading confidence	Sex	Adjusted R^2
Length 1 YO	356	**2.18**	0.45	**2.02**	**inter**	**inter**	**inter**	0.84	0.32	2.62	*27.0%*
Length 2 YO	339	**2.23**	0.85	**2.31**	**inter**	**inter**	**inter**	0.00	1.37	**5.21**	*26.1%*
Length 3 YO	231	1.80	0.38	**2.11**	**inter**	**inter**	**14.53 (2.11)**	0.89	3.56	**6.99**	*30.0%*
Length 4 YO	142	**3.56**	1.16	**1.88**	**inter**	**inter**	**inter**	**3.97 (-0.54)**	0.10	1.47	*36.1%*
Growth 0-1 YO	356	**2.18**	0.45	**2.02**	**inter**	**inter**	**inter**	0.84	0.32	2.62	*27.0%*
Growth 1-2 YO	339	0.49	0.68	**2.76**	**inter**	**inter**	1.70	0.92	**7.64**	**5.76**	*20.4%*
Growth 2-3 YO	231	0.59	0.76	1.43	**2.71**	0.13	1.97	0.45	**5.42 (0.22)**	0.68	*6.8%*
Growth 3-4 YO	142	1.87	1.28	1.69	**3.32**	1.97	1.58	0.43	0.04	0.06	*11.6%*

Estimates are provided for significant continuous variables that are not in an interaction. Removal of variables that are in an interaction was not tested, thus we provide no value in these cases and indicate them with the term "inter".

Annexes Chapitre 3

Appendix A: Supplementary analyses and information on methods and results.

Table S3.A1 Final models after selection of generalized linear mixed models when considering external and internal parasites for individual analyses of *Salvelinus fontinalis*.

Dependent variables	Distribution	*n*	Fixed factors	Estimate	S.E.M.	*z*	*P*
Infection status							
External parasites	Binomial	1240	Total length	0.74	0.14	5.46	< 0.001
Marginal R^2 = 0.004							
Conditional R^2 = 0.972							
Internal parasites	Binomial	1240	Total length	0.65	0.12	5.49	< 0.001
Marginal R^2 = 0.040			Sex (indeterminate)	–0.74	0.37	2.01	< 0.05
Conditional R^2 = 0.812			Sex (male)	0.36	0.20	1.81	> 0.05
Intensity of infection							
External parasites	Binomial	482	Total length	0.83	0.16	5.19	< 0.001
Marginal R^2 = 0.085							
Conditional R^2 = 0.596							
Internal parasites	Binomial	459	Total length	1.56	0.22	7.06	< 0.001
Marginal R^2 = 0.143							
Conditional R^2 = 0.805							

Lake and wildlife reserve were used as random factors. In (b) the infection status model, sex has three levels and females are the reference level. R^2 presented here are estimated with the package piecewiseSEM.

Table S3.A2 Final models after selection of generalized linear mixed models for prevalence analyses of external and internal parasites in populations of *Salvelinus fontinalis*.

Dependent variables	Distribution	n	Fixed factors	Estimate	S.E.M.	*z*	*P*
External parasites	Binomial	28	Lake area	0.20	0.10	2.07	0.039
Marginal R^2 = 0.085			Brook charr density	0.19	0.09	2.02	0.044
Conditional R^2 = 0.293			Mean *q*-value	–0.36	0.10	3.79	<0.001
			Mean total length	–0.40	0.09	4.37	<0.001
			Mean Fulton index	0.25	0.12	2.13	0.033
Internal parasites	Binomial	28	Lake area	0.45	0.10	4.68	<0.001
Marginal R^2 = 0.253			Brook charr density	0.55	0.09	5.90	<0.001
Conditional R^2 = 0.319			White sucker density	–0.24	0.09	2.67	0.008
			Mean *q*-value	–0.80	0.10	7.79	<0.001
			Mean Fulton index	0.31	0.11	2.71	0.007

Wildlife reserve was used as a random factor. R^2 presented here are estimated with the package piecewiseSEM.

Table S3.A3 Characteristics of microsatellite loci used to genotype *Salvelinus fontinalis* with their names, repeat motifs, allele size range, $MgCl_2$ concentration (mM) used in PCRs, annealing temperature and number of cycles used for PCR amplification and the references for further details about loci.

Locus	Repeat motif	Range (bp)	$MgCl_2$ (mM)	Annealing Temperature ($_oC$)	Number of cycles	Source
Sfo-12	$(GT)_5CC(GT)_{10}CC(GT)_{15}$	244–273	1.5	58	35	Angers *et al.*, 1995
Sco218	$(GATA)_{31}$	130–214	1	56	35	Dehaan & Ardren, 2005
Sco216	$(CAGT)_{18}(CAGG)_{10}$	117–201	1.2	58	30	Dehaan & Ardren, 2005
*Sfo*B52	$(GCGT)_{12}$	189–229	1.2	60	35	King *et al.*, 2012
*Sfo*C24	$(GAT)_{10}$	103–124	1	58	30	King *et al.*, 2012
*Sfo*C86	$(GAT)_8$	91–124	1.5	58	30	King *et al.*, 2012
*Sfo*C129	$(GAT)_8$	205–238	1	60	35	King *et al.*, 2012
*Sfo*C113	$(GAT)_{12}$	125–155	1.2	56	35	King *et al.*, 2012
*Sfo*C88	$(GAT)_{16}$	167–194	1	54	35	King *et al.*, 2012
*Sfo*D75	$(TAGA)_{17}$	160–228	1	58	35	King *et al.*, 2012
*Sfo*D100	$(TAGA)_{11}$	197–245	1.2	58	35	King *et al.*, 2012
*Sfo*D91	$(TAGA)_{13}$	204–300	1	56	35	King *et al.*, 2012
*Sfo*C115	$(CTCA)_{21}$	218–347	1.2	60	35	King *et al.*, 2012
Ssa85	$(GT)_{14}$	95–133	1	60	35	O'Reilly *et al.*, 1996
Ssa197	$(GT)_5C(TG)_4TC(TG)_3A(GTGA)_{15}$	138–158	1	62	35	O'Reilly *et al.*, 1996
Sfo269Lav	$(CA)_{28}$	272–332	1.5	48	35	Perry *et al.*, 2005
Sfo262Lav	$(TG)_{20}(CGTG)_7CGCG(CGTG)_2(CG)_3$	256–316	1.2	59	35	Perry *et al.*, 2005
Sfo226Lav	$(TG)_{21}(CGTG)_{13}$	335–389	1	60	35	Perry *et al.*, 2005

Locus	Repeat motif	Range (bp)	$MgCl_2$ (mM)	Annealing Temperature ($_oC$)	Number of cycles	Source
Sfo266Lav	$TGCG(TG)_{13}N_{16}(TG)_3TGCG$	241–341	1.2	56	35	Perry *et al.*, 2005
*One*µ8	$(CA)_{24}$	155–169	1.5	60	35	Scribner *et al.*, 1996

Table S3.A4 Prevalence (% of infected individuals) found for each parasite species and for external, internal and total parasites of *Salvelinus fontinalis* in the sampled lakes.

Sampling year	Reserve	Lake	External parasites			Internal parasites					Total	Total all parasites
						Ancantho-cephala	*Cestoda*			*Nema-todes*		
			Gill lice *Salmincola*	Cysts *Hetero-phyidae*	*Total*	*Echino-rhynchus*	subclass *Eucestoda* *Eubothrium* or *Proteo-cephalus*	*Diphyllo-bothrium*	*Ligula*	*Metabro-nema*		
2015	MAS	DEM	54.55	0	54.55	96.97	0	0	0	0	96.97	96.97
2015	MAS	HEAD	86.96	2.17	86.96	100	17.39	0	0	0	100	100
2015	MAS	CER	74.63	0	74.63	98.51	35.82	4.48	0	0	98.51	100
2015	MAS	CHAMB	65.85	9.76	68.29	19.51	0	0	0	0	19.51	80.49
2015	MAS	DETP	0	0	0	0	0	0	0	0	0	0
2015	MAS	PIT	0	0	0	0	0	0	0	0	0	0
2015	PN	SOR	39.13	84.78	86.96	0	23.91	0	0	0	23.91	86.96
2015	PN	LANG	53.66	80.49	85.37	0	19.51	0	0	0	19.51	85.37
2015	PN	MDF	71.05	0	71.05	60.53	42.11	13.16	0	0	76.32	92.11
2015	PN	CAR	0	81.25	81.25	0	3.13	0	0	0	3.13	81.25
2015	STM	CORB	0	0	0	100	0	4.65	0	0	100	100
2015	STM	BRO	0	22.22	22.22	0	52.38	4.76	1.59	0	52.38	58.73
2016	STM	COUR	0	0	0	0	0	2.5	0	52.5	52.5	52.5
2015	STM	PORT	0	0	0	0	0	22.5	0	0	22.5	22.5
2016	STM	CLAI	97.5	0	97.5	0	5	0	0	17.5	22.5	100
2015	STM	MIL	75	84.09	93.18	0	0	2.27	0	0	2.27	93.18

Sampling year	Reserve	Lake	External parasites			Internal parasites					Total	Total all parasites
						Ancantho-cephala	*Cestoda*			*Nema-todes*		
			Gill lice *Salmincola*	Cysts *Hetero-phyidae*	*Total*	*Echino-rhynchus*	subclass *Eucestoda* *Eubothrium* or *Proteo-cephalus*	*Diphyllo-bothrium*	*Ligula*	*Metabro-nema*		
2016	STM	EPER	0	0	0	0	0	0	0	2.56	2.56	2.56
2016	STM	PLON	0	0	0	0	0	30	0	0	30	30
2016	STM	ECAR	0	0	0	0	0	0	0	2.56	2.56	2.56
2015	STM	SOU	56.1	48.78	63.41	2.44	43.9	7.32	9.76	0	53.66	70.73
2016	STM	EST	0	0	0	0	0	7.69	0	74.36	76.92	76.92
2015	STM	PER	0	100	100	0	0	0	0	0	0	100
2016	STM	BEC	0	0	0	0	0	25	0	2.5	25	25
2016	STM	SUD	0	0	0	0	0	15.38	0	56.41	61.54	61.54
2016	STM	PIN	60.94	0	60.94	0	0	0	0	0	0	60.94
2016	STM	BOUCH	0	0	0	0	0	22.5	0	0	22.5	22.5
2016	STM	CARD	0	0	0	0	0	2.56	0	0	2.56	2.56
2016	STM	HAM	0	0	0	0	0	0	0	0	0	0

STM, Saint-Maurice; MAS, Mastigouche; PN, Portneuf. The complete names of lakes can be found in Table S3.1.

Table S3.A5 Values of expected (H_E) and observed (H_O) heterozygozity for *Salvelinus fontinalis* in each lake and domestic strains.

Sampling year	Reserve	Lake	H_E	H_O
2015	MAS	DEM	0.587	0.564
2015	MAS	HEAD	0.630	0.633
2015	MAS	CER	0.582	0.566
2015	MAS	CHAMB	0.748	0.740
2015	MAS	DETP	0.690	0.813
2015	MAS	PIT	0.704	0.811
2015	PN	SOR	0.588	0.566
2015	PN	LANG	0.553	0.512
2015	PN	MDF	0.457	0.464
2015	PN	CAR	0.645	0.617
2015	STM	CORB	0.598	0.573
2015	STM	BRO	0.652	0.644
2016	STM	COUR	0.667	0.815
2015	STM	PORT	0.521	0.528
2016	STM	CLAI	0.651	0.658
2015	STM	MIL	0.718	0.701
2016	STM	EPER	0.684	0.806
2016	STM	PLON	0.695	0.765
2016	STM	ECAR	0.713	0.728
2015	STM	SOU	0.691	0.654
2016	STM	EST	0.684	0.683
2015	STM	PER	0.644	0.640
2016	STM	BEC	0.699	0.725
2016	STM	SUD	0.717	0.704
2016	STM	PIN	0.721	0.777
2016	STM	BOUCH	0.706	0.783
2016	STM	CARD	0.713	0.765
2016	STM	HAM	0.713	0.823
2007	NA	ECO	0.654	0.630
2007	NA	BOU	0.492	0.494
2007-2016	NA	A	0.709	0.783
2007	NA	JC	0.736	0.710

STM, Saint-Maurice; MAS, Mastigouche; PN, Portneuf. The complete names of lakes can be found in Table S3.1.

Appendix B: References

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Annexes Chapitre 4

Appendix A: Supplementary analyses and information on methods and results.

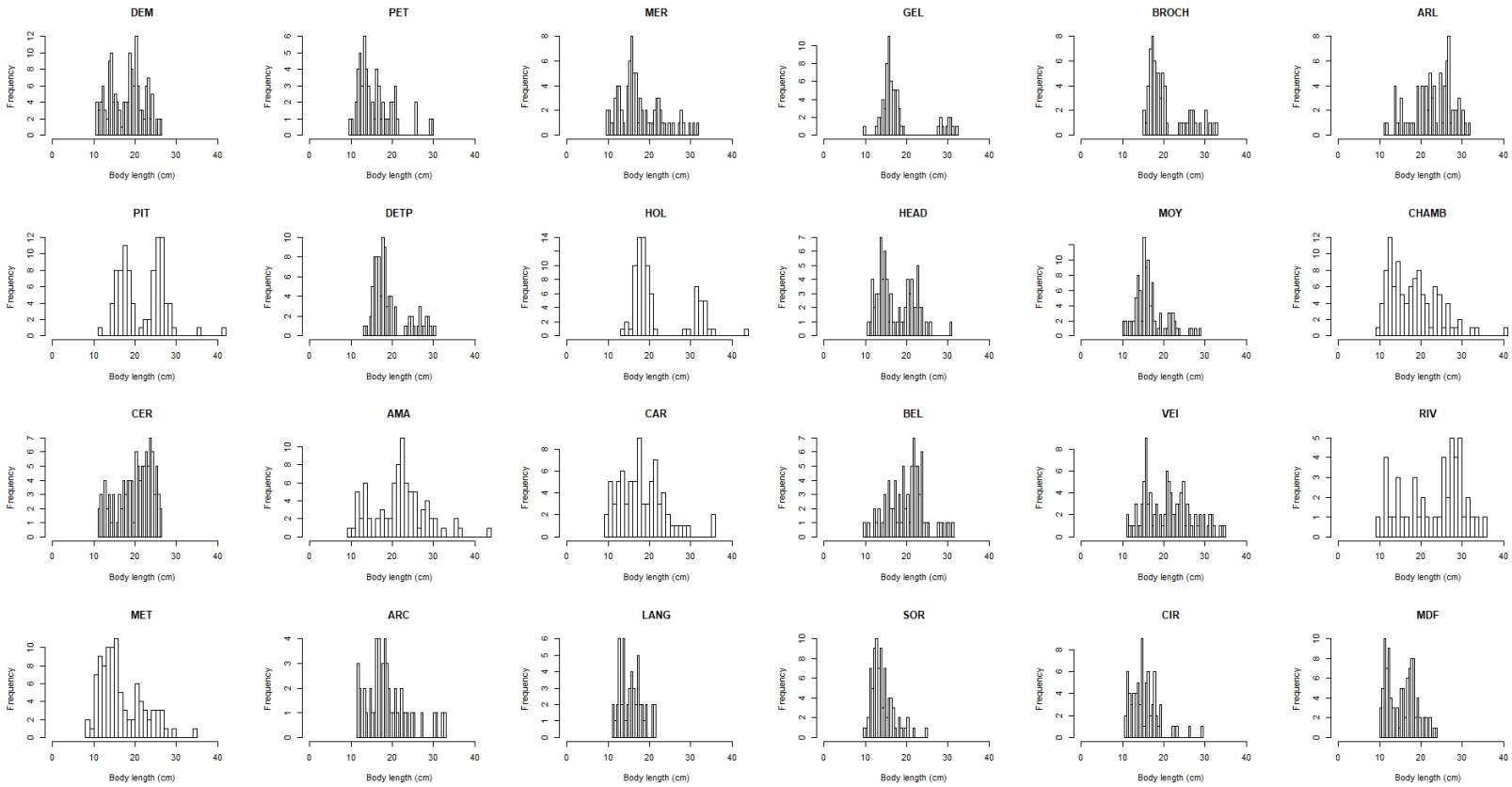

Figure S4.A1 Distribution of body length (length from the snout to the base of the caudal fin, cm) of sampled fish in each lake sampled in 2007-2008.

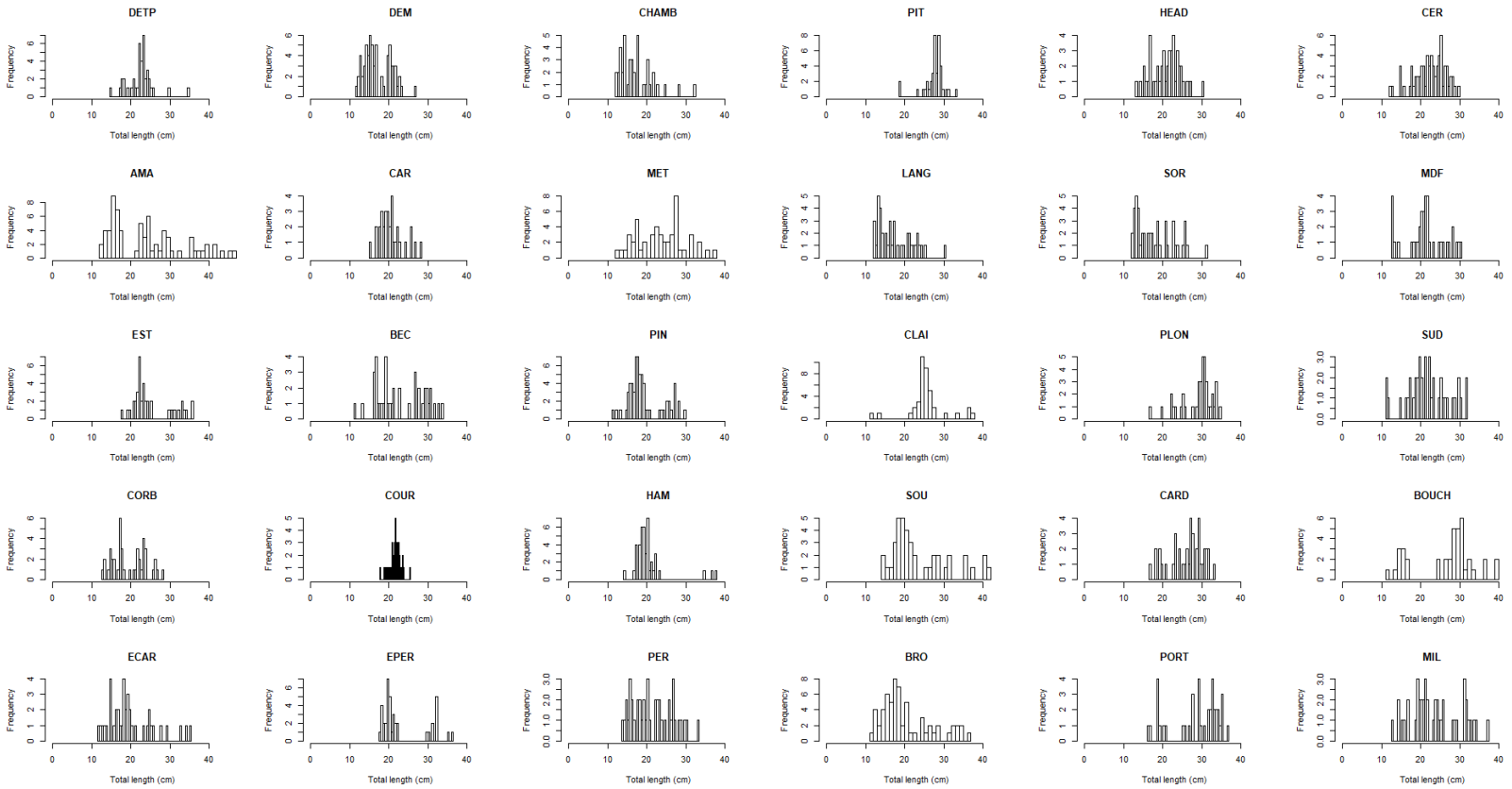

Figure S4.A2 Distribution of total length (cm) of sampled fish in cm in each lake sampled in 2014-2016.

Table S4.A1 Characteristics of microsatellite loci used to genotype brook charr (*Salvelinus fontinalis*).

Locus	Repeat motif (bp)	Range	[$MgCl_2$] (mM)	Annealing temperature	Number of cycles	Source
Sfo-12	$(GT)_5CC(GT)_{10}CC(GT)_{15}$	244-273	1.5	58°C	35	Angers et al. 1995
Sco218	$(GATA)_{31}$	130-214	1	56°C	35	Dehaan and Ardren 2005
Sco216	$(CAGT)_{18}(CAGG)_{10}$	117-201	1.2	58°C	30	Dehaan and Ardren 2005
*Sfo*B52	$(GCGT)_{12}$	189-229	1.2	60°C	35	King et al. 2012
*Sfo*C24	$(GAT)_{10}$	103-124	1	58°C	30	King et al. 2012
*Sfo*C86	$(GAT)_8$	91-124	1.5	58°C	30	King et al. 2012
*Sfo*C129	$(GAT)_8$	205-238	1	60°C	35	King et al. 2012
*Sfo*C113	$(GAT)_{12}$	125-155	1.2	56°C	35	King et al. 2012
*Sfo*C88	$(GAT)_{16}$	167-194	1	54°C	35	King et al. 2012
*Sfo*D75	$(TAGA)_{17}$	160-228	1	58°C	35	King et al. 2012
*Sfo*D100	$(TAGA)_{11}$	197-245	1.2	58°C	35	King et al. 2012
*Sfo*D91	$(TAGA)_{13}$	204-300	1	56°C	35	King et al. 2012
*Sfo*C115	$(CTCA)_{21}$	218-347	1.2	60°C	35	King et al. 2012
Ssa85	$(GT)_{14}$	95-133	1	60°C	35	O'Reilly et al. 1996
Ssa197	$(GT)_5C(TG)_4TC(TG)_3A(GTGA)_{15}$	138-158	1	62°C	35	O'Reilly et al. 1996
Sfo269Lav	$(CA)_{28}$	272-332	1.5	48°C	35	Perry et al. 2005
Sfo262Lav	$(TG)_{20}(CGTG)_7CGCG(CGTG)_2(CG)_3$	256-316	1.2	59°C	35	Perry et al. 2005
Sfo226Lav	$(TG)_{21}(CGTG)_{13}$	335-389	1	60°C	35	Perry et al. 2005
Sfo266Lav	$TGCG(TG)_{13}N_{16}(TG)_3TGCG$	241-341	1.2	56°C	35	Perry et al. 2005
*One*µ8	$(CA)_{24}$	155-169	1.5	60°C	35	Scribner et al. 1996

All loci were used to genotype 2014-2016 individuals and all loci but *Sco216* were used to genotype 2007-2008 individuals.

Table S4.A2 Correlations between the 3 methods of Ne estimation with the temporal method.

	Jorde-Ryman	Nei-Tajima
Nei-Tajima	0.94 [0.79-0.98] p < 0.001	/
Pollack	0.88 [0.63-0.97] p < 0.001	0.99 [0.96-1.00] p < 0.001

Degrees of freedom for each correlation is 10.

Table S4.A3 Pearson correlations between the parameters of intensity of stocking.

	Number of stocking events	Number of fish stocked/ha	Proportion of domestic genes
Number of fish stocked/ha	0.59 [0.36 - 0.76] p < 0.001	/	/
Proportion of domestic genes	0.58 [0.34 - 0.75] p < 0.001	0.59 [0.36 - 0.76] p<0.001	/
Number of years since last stocking event	-0.56 [-0.73 - -0.31] p < 0.001	-0.36 [-0.59 - -0.07] p = 0.02	-0.48 [-0.68 - -0.21] p = 0.001

Degrees of freedom for each correlation is 42.

Table S4.A4 Summary of effective population size (Ne) estimates obtained with all methods and their respective thresholds of rare alleles exclusion (PCrit).

Population	Reserve	Ne_{SIB} ± 95% CI	Ne_{LD} ± 95% CI Pcrit=0.05	Ne_{MT} ± 95% CI JR Method Pcrit=0.05	Ne_{MT} ± 95% CI NT Method Pcrit=0.05	Ne_{MT} ± 95% CI Pk Method Pcrit=0.05	Ne_{LD} ± 95% CI Pcrit=0.02	Ne_{MT} ± 95% CI JR Method Pcrit=0.02	Ne_{MT} ± 95% CI NT Method Pcrit=0.02	Ne_{MT} ± 95% CI Pk Method Pcrit=0.02
07-08_DEM	MAS	48.0 [32.0 - 71.0]	14.7 [11.2 - 18.8]	NA	NA	NA	19.9 [15.8 - 24.8]	NA	NA	NA
07-08_PET	MAS	43.0 [28.0 - 70.0]	29.0 [24.4 - 35.0]	NA	NA	NA	37.3 [31.9 - 44.2]	NA	NA	NA
07-08_MER	MAS	59.0 [41.0 - 85.0]	31.4 [26.1 - 38.1]	NA	NA	NA	43.7 [38.0 - 50.6]	NA	NA	NA
07-08_GEL	MAS	30.0 [20.0 - 50.0]	32.7 [26.1 - 41.5]	NA	NA	NA	41.5 [34.9 - 49.9]	NA	NA	NA
07-08_BROCH	MAS	31.0 [20.0 - 52.0]	34.0 [27.4 - 42.8]	NA	NA	NA	42.4 [35.5 - 51.4]	NA	NA	NA
07-08_ARL	MAS	28.0 [18.0 - 46.0]	40.5 [27.1 - 64.5]	NA	NA	NA	47.1 [32.1 - 74.1]	NA	NA	NA
07-08_PIT	MAS	63.0 [44.0 - 92.0]	57.0 [44.1 - 76.1]	NA	NA	NA	77.1 [63.2 - 96.3]	NA	NA	NA
07-08_DETP	MAS	58.0 [39.0 - 82.0]	74.3 [61.0 - 92.5]	NA	NA	NA	80.4 [67.6 - 97.4]	NA	NA	NA
07-08_HOL	MAS	59.0 [40.0 - 83.0]	79.0 [62.0 - 104.7]	NA	NA	NA	87.1 [71.2 - 109.8]	NA	NA	NA
07-08_HEAD	MAS	62.0 [43.0 - 90.0]	79.3 [55.7 - 124.7]	NA	NA	NA	122.6 [88.9 - 186.8]	NA	NA	NA
07-08_MOY	MAS	35.0 [23.0 - 56.0]	84.6 [37.0 - 504.1]	NA	NA	NA	74.7 [36.1 - 260.1]	NA	NA	NA
07-08_CHAMB	MAS	107.0 [77.0 - 145.0]	124.5 [96.3 - 169.5]	NA	NA	NA	152.4 [119.3 - 204.8]	NA	NA	NA
07-08_CER	MAS	NA	181.0 [103.0 - 492.0]	NA	NA	NA	209.1 [139.9 - 375.9]	NA	NA	NA

Population	Reserve	Ne_{SIB} ± 95% CI	Ne_{LD} ± 95% CI Pcrit=0.05	Ne_{MT} ± 95% CI JR Method Pcrit=0.05	Ne_{MT} ± 95% CI NT Method Pcrit=0.05	Ne_{MT} ± 95% CI Pk Method Pcrit=0.05	Ne_{LD} ± 95% CI Pcrit=0.02	Ne_{MT} ± 95% CI JR Method Pcrit=0.02	Ne_{MT} ± 95% CI NT Method Pcrit=0.02	Ne_{MT} ± 95% CI Pk Method Pcrit=0.02
14-16_DETP	MAS	25.0 [15.0 - 44.0]	42.3 [31.8 - 59.5]	20.7 [14.4 - 36.4]	21.9 [15.5 - 30.7]	20.4 [14.4 - 28.7]	34.3 [28.2 - 42.7]	21.2 [15.4 - 33.9]	23 [17.9 - 29.4]	21.8 [17.1 - 27.5]
14-16_DEM	MAS	47.0 [31.0 - 71.0]	50.8 [30.7 - 100.7]	56.9 [28.1 - Inf]	86.2 [37.5 - 248.2]	94.0 [42.7 - 261.7]	68.7 [43.8 - 128.1]	56.6 [28.2 - Inf]	87.7 [41.5 - 221.3]	94.9 [47.6 - 224.7]
14-16_CHAMB	MAS	49.0 [31.0 - 77.0]	54.4 [42.4 - 73.4]	61.4 [37.8 - 162.3]	72.8 [45.9 - 125.6]	72.1 [46.6 - 120.0]	65.3 [52.6 - 84.2]	61.8 [38.3 - 159.0]	77.7 [48.3 - 138.1]	76.9 [48.0 - 135.8]
14-16_PIT	MAS	48.0 [30.0 - 74.0]	58.9 [43.3 - 87.3]	30.2 [21.6 - 50.4]	29.4 [20.4 - 42.5]	27.5 [18.6 - 40.7]	61.9 [49.9 - 79.6]	33.4 [23.7 - 56.6]	35.5 [25.7 - 49.4]	34.0 [24.2 - 48.1]
14-16_HEAD	MAS	49.0 [31.0 - 76.0]	87.8 [58.2 - 160.1]	278.4 [85.9 - Inf]	229.8 [80.8 - Inf]	187.1 [73.8 - Inf]	126.8 [85.8 - 226.6]	533.4 [121.6 - Inf]	236.0 [100.7 - 6909.8]	195.9 [91.8 - 1089.8]
14-16_CER	MAS	61.0 [42.0 - 89.0]	151.9 [85.6 - 456.6]	Inf [318.0 - Inf]	Inf [177.6 - Inf]	Inf [147.2 - Inf]	166.2 [108.5 - 321.3]	1879.7 [226.2 - Inf]	409.4 [131.9 - Inf]	309.7 [114.4 - Inf]
07-08_AMA	PN	11.0 [6.0 - 26.0]	2.9 [2.6 - 3.1]	NA	NA	NA	8.0 [7.0 - 9.0]	NA	NA	NA
07-08_CAR	PN	31.0 [20.0 - 51.0]	8.8 [7.2 - 10.5]	NA	NA	NA	8.9 [7.8 - 10.0]	NA	NA	NA
07-08_BEL	PN	39.0 [25.0 - 62.0]	13.4 [11.9 - 15.0]	NA	NA	NA	24.1 [21.9 - 26.6]	NA	NA	NA
07-08_VEI	PN	52.0 [36.0 - 74.0]	18.2 [15.5 - 21.2]	NA	NA	NA	30.4 [26.4 - 35.1]	NA	NA	NA
07-08_RIV	PN	38.0 [24.0 - 62.0]	22.6 [18.6 - 27.9]	NA	NA	NA	30.5 [25.3 - 37.4]	NA	NA	NA
07-08_MET	PN	70.0 [50.0 - 100.0]	36.9 [32.2 - 42.5]	NA	NA	NA	54.3 [48.0 - 61.7]	NA	NA	NA
07-08_ARC	PN	40.0 [25.0 - 63.0]	41.6 [29.5 - 62.9]	NA	NA	NA	38.6 [28.6 - 54.5]	NA	NA	NA
07-08_LANG	PN	24.0 [14.0 - 44.0]	59.2 [37.0 - 119.9]	NA	NA	NA	118.0 [65.8 - 394.9]	NA	NA	NA

Population	Reserve	Ne_{SIB} ± 95% CI	Ne_{LD} ± 95% CI Pcrit=0.05	Ne_{MT} ± 95% CI JR Method Pcrit=0.05	Ne_{MT} ± 95% CI NT Method Pcrit=0.05	Ne_{MT} ± 95% CI Pk Method Pcrit=0.05	Ne_{LD} ± 95% CI Pcrit=0.02	Ne_{MT} ± 95% CI JR Method Pcrit=0.02	Ne_{MT} ± 95% CI NT Method Pcrit=0.02	Ne_{MT} ± 95% CI Pk Method Pcrit=0.02
07-08_SOR	PN	60.0 [42.0 - 86.0]	99.4 [69.4 - 159.3]	NA	NA	NA	114.2 [84.2 - 168.3]	NA	NA	NA
07-08_CIR	PN	61.0 [43.0 - 89.0]	105.6 [71.5 - 181.7]	NA	NA	NA	129.8 [94.8 - 195.9]	NA	NA	NA
07-08_MDF	PN	NA	2503.0 [282.4 - Inf]	NA	NA	NA	Inf [404.3 - Inf]	NA	NA	NA
14-16_AMA	PN	15.0 [8.0 - 30.0]	3.9 [3.5 - 5.2]	8.7 [6.5 - 13.2]	9.4 [7.1 - 12.1]	9.0 [6.9 - 11.4]	4.9 [3.9 - 6.3]	9.4 [7.1 - 14.0]	12.3 [9.8 - 15.2]	12.2 [10.0 - 14.8]
14-16_CAR	PN	26.0 [16.0 - 47.0]	20.8 [16.0 - 27.8]	56.5 [33.5 - 180.3]	54.0 [30.6 - 108.8]	49.4 [27.5 - 99.8]	19.5 [16.3 - 23.5]	55.1 [34.5 - 135.8]	54.0 [35.8 - 86.8]	50.2 [33.1 - 80.7]
14-16_MET	PN	41.0 [26.0 - 64.0]	35.8 [30.5 - 42.4]	95.0 [48.2 - 3422.4]	127.5 [54.9 - 613.0]	126.5 [54.5 - 596.4]	53.2 [46.2 - 62.0]	90.0 [47.2 - 974.0]	132.2 [66.9 - 385.4]	131.7 [67.6 - 368.9]
14-16_LANG	PN	39.0 [25.0 - 67.0]	91.1 [49.7 - 308.2]	35.0 [19.8 - 149.2]	41.2 [24.0 - 78.0]	40.4 [24.4 - 72.6]	138.5 [75.3 - 544.0]	34.9 [19.8 - 145.8]	42.5 [25.1 - 78.8]	42.0 [25.8 - 74.1]
14-16_SOR	PN	NA	377.9 [131.4 - Inf]	71.4 [31.2 - Inf]	73.7 [32.4 - 241.6]	68.5 [31.8 - 193.0]	270.8 [122.1 - Inf]	71.6 [31.5 - Inf]	85.8 [38.5 - 290.0]	83.3 [38.8 - 253.7]
14-16_MDF	PN	NA	538.4 [92.9 - Inf]	280.5 [53.4 - Inf]	192.2 [28.5 - Inf]	221.6 [31.2 - Inf]	132.4 [56.4 - Inf]	232 [52.1 - Inf]	152.4 [35.2 - Inf]	155.2 [38.0 - Inf]
14-16_EST	STM	43.0 [27.0 - 72.0]	12.6 [10.4 - 15.3]	NA	NA	NA	18.4 [15.9 - 21.3]	NA	NA	NA
14-16_BEC	STM	24.0 [14.0 - 43.0]	20.5 [17.4 - 24.4]	NA	NA	NA	28.5 [24.3 - 33.9]	NA	NA	NA
14-16_PIN	STM	31.0 [20.0 - 52.0]	26.9 [23.4 - 31.2]	NA	NA	NA	40.7 [35.2 - 47.6]	NA	NA	NA
14-16_CLAI	STM	35.0 [22.0 - 56.0]	30.8 [23.9 - 41.0]	NA	NA	NA	24.6 [20.3 - 30.1]	NA	NA	NA
14-16_PLON	STM	44.0 [28.0 - 69.0]	31.4 [24.6 - 41.5]	NA	NA	NA	39.6 [31.9 - 50.8]	NA	NA	NA

Population	Reserve	Ne_{SIB} ± 95% CI	Ne_{LD} ± 95% CI Pcrit=0.05	Ne_{MT} ± 95% CI JR Method Pcrit=0.05	Ne_{MT} ± 95% CI NT Method Pcrit=0.05	Ne_{MT} ± 95% CI Pk Method Pcrit=0.05	Ne_{LD} ± 95% CI Pcrit=0.02	Ne_{MT} ± 95% CI JR Method Pcrit=0.02	Ne_{MT} ± 95% CI NT Method Pcrit=0.02	Ne_{MT} ± 95% CI Pk Method Pcrit=0.02
14-16_SUD	STM	52.0 [34.0 - 84.0]	36.7 [29.0 - 48.2]	NA	NA	NA	73.0 [56.2 - 101.0]	NA	NA	NA
14-16_CORB	STM	36.0 [22.0 - 58.0]	39.9 [26.7 - 67.1]	NA	NA	NA	50.0 [35.2 - 78.8]	NA	NA	NA
14-16_COUR	STM	57.0 [38.0 - 88.0]	42.2 [30.6 - 63.0]	NA	NA	NA	53.9 [39.4 - 80.0]	NA	NA	NA
14-16_HAM	STM	30.0 [19.0 - 51.0]	48.8 [37.4 - 67.2]	NA	NA	NA	84.7 [67.1 - 112.3]	NA	NA	NA
14-16_SOU	STM	42.0 [26.0 - 67.0]	54.9 [41.4 - 77.4]	NA	NA	NA	49.7 [40.3 - 63.3]	NA	NA	NA
14-16_CARD	STM	48.0 [29.0 - 76.0]	57.2 [43.1 - 81.4]	NA	NA	NA	74.9 [59.1 - 99.9]	NA	NA	NA
14-16_BOUCH	STM	54.0 [35.0 - 83.0]	62.6 [44.5 - 98.3]	NA	NA	NA	82.1 [64.3 - 110.8]	NA	NA	NA
14-16_ECAR	STM	45.0 [28.0 - 71.0]	66.2 [46.5 - 106.4]	NA	NA	NA	72.5 [54.6 - 103.7]	NA	NA	NA
14-16_EPER	STM	56.0 [36.0 - 85.0]	80.6 [51.2 - 162.4]	NA	NA	NA	74.9 [54.1 - 115.2]	NA	NA	NA
14-16_PER	STM	48.0 [30.0 - 77.0]	86.8 [54.3 - 182.5]	NA	NA	NA	106.5 [75.0 - 174.0]	NA	NA	NA
14-16_BRO	STM	73.0 [50.0 - 104.0]	94.0 [66.0 - 150.5]	NA	NA	NA	106.5 [77.3 - 161.9]	NA	NA	NA
14-16_PORT	STM	NA	153.4 [74.5 - 2043.4]	NA	NA	NA	228.5 [110.3 - 8451.7]	NA	NA	NA
14-16_MIL	STM	NA	2198.1 [261.0 - Inf]	NA	NA	NA	644.3 [236.1 - Inf]	NA	NA	NA

Abbreviations: STM = Saint-Maurice, MAS = Mastigouche, PN = Portneuf. “NA” is for non-available data

Table S4.A5 Final model (GLM, negative binomial distribution) obtained after backward selection to assess the relationship between stocking status (stocked VS unstocked lakes) impacts effective population size (Ne_{LD}, estimated with the linkage disequilibrium method with PCrit=0.02).

Dependent variable	n	Fixed factors	Estimate	Standard error	z-value	p-value
Ne_{LD}	54	Intercept	4.64	0.18	25.58	< 0.001
R^2 = 0.289		Reserve Mastigouche	-0.51	0.26	-1.95	0.051
		Reserve Portneuf	-0.82	0.28	-2.92	< 0.001
		Stocking status Unstocked	1.42	0.29	4.90	< 0.001
		Variable removed:	Lake area (p = 0.736)			

Table S4.A6 Final models (GLM, negative binomial distribution) assessing whether stocking intensity is related to effective population size (Ne_{LD} estimated with the linkage disequilibrium method, PCrit=0.02).

Dependent variable	n	Fixed factors	Estimate	SE	z-value	*p*
Final model with non-significant intensity of stocking variables						
Ne_{LD}	44	Intercept	4.26	0.2	21.82	<0.001
R^2=0.195		Reserve Mastigouche	-0.38	0.26	-1.43	0.153
		Reserve Portneuf	-1.04	0.29	-3.56	<0.001
		Variables removed:	Lake area (p≥0.650)			
			Number of stocking events (p=0.319)			
			Number of fish stocked/ha (p=0.683)			
			Number of years since last stocking (p=0.089)			
			Mean q-value without lake 14-16_MIL (p=0.203)*			
Final model with significant proportion of domestic background (mean q-value)						
Ne_{LD}	44	Intercept	4.26	0.19	22.736	<0.001
R^2=0.263		Reserve Mastigouche	-0.40	0.26	-1.55	0.121
		Reserve Portneuf	-1.19	0.29	-4.11	<0.001
		Mean q-value	-0.24	0.12	-2.068	0.039
		Variable removed:	Lake area (p=0.699)			
Final model with significant number of years since last stocking without lake 14-16_MIL						
Ne_{LD}	43	Intercept	4.21	0.14	29.22	< 0.001
R^2 = 0.248		Reserve Mastigouche	0.03	0.21	0.15	0.880
		Reserve Portneuf	-0.59	0.23	-2.52	0.012
		Number of years since last stocking without lake 14-16_MIL	0.23	0.09	2.480	0.013
		Variable removed:	Lake area (p= 0.547)			

* When the population 14-16_MIL was removed, mean q-value was no longer significant. R^2 are from final models.

Table S4.A7 Final model (GLM, negative binomial distribution) obtained after backward selection to assess the relationship between stocking status (stocked VS unstocked lakes) impacts effective population size (Ne_{LD}, estimated with the linkage disequilibrium method with PCrit=0.05) after the removal of populations having a strong linkage disequilibrium.

Dependent variable	n	Fixed factors	Estimate	Standard error	z-value	p-value
Ne_{LD}	51	Intercept	5.16	0.25	20.95	< 0.001
R^2 = 0.351		Reserve Mastigouche	2.01	0.41	4.93	< 0.001
		Reserve Portneuf	-1.30	0.36	-3.64	< 0.001
		Stocking status Unstocked	-1.13	0.41	-2.74	0.01
		Variable removed:	Lake area (p = 0.757)			

Table S4.A8 Final model (GLM, negative binomial distribution) obtained after backward selection to assess the relationship between stocking status (stocked VS unstocked lakes) impacts effective population size (Ne_{LD}, estimated with the linkage disequilibrium method with PCrit=0.02) after the removal of populations having a strong linkage disequilibrium.

Dependent variable	n	Fixed factors	Estimate	Standard error	z-value	p-value
Ne_{LD}	51	Intercept	4.39	0.12	37.46	< 0.001
$R^2 = 0.560$		Stocking status Unstocked	0.92	0.26	3.48	< 0.001
		Variable removed:	Lake area (p = 0.731)			
			Reserve identity (p=0.095)			

Table S4.A9 Final models (GLM, negative binomial distribution) assessing whether stocking intensity is related to effective population size (Ne_{LD} estimated with the linkage disequilibrium method, PCrit=0.05) after the removal of populations having a strong linkage disequilibrium.

Dependent variable	n	Fixed factors	Estimate	SE	z-value	*p*
Final model with non-significant intensity of stocking variables						
Ne_{LD}	41	Intercept	5.16	0.23	21.99	<0.001
R^2=0.262		Reserve Mastigouche	-1.13	0.35	-3.22	0.001
		Reserve Portneuf	-1.55	0.43	-3.64	<0.001
		Variables removed:	Lake area (p≥0.881)			
			Number of stocking events (p=0.621)			
			Number of fish stocked/ha (p=0.190)			
			Number of years since last stocking (p=0.495)			
Final model with significant proportion of domestic background (mean q-value)						
Ne_{LD}	41	Intercept	5.06	0.22	22.64	<0.001
R^2=0.362		Reserve Mastigouche	-0.96	0.33	-2.91	0.004
		Reserve Portneuf	-1.74	0.42	-4.16	<0.001
		Mean q-value	-0.45	0.16	-2.91	0.004
		Variable removed:	Lake area (p=0.618)			

Table S4.A10 **Final models (GLM, negative binomial distribution) assessing whether stocking intensity is related to effective population size (Ne_{LD} estimated with the linkage disequilibrium method, PCrit=0.02) after the removal of populations having a strong linkage disequilibrium.**

Dependent variable	n	Fixed factors	Estimate	SE	z-value	*p*
Final model with non-significant intensity of stocking variables						
Ne_{LD}	41	Intercept	4.64	0.17	27.92	<0.001
R^2=0.131		Reserve Mastigouche	-0.38	0.25	-1.53	0.126
		Reserve Portneuf	-0.77	0.30	-2.56	0.010
		Variables removed:	Lake area (*p*≥0.714)			
			Mean q-value (p=0.055)			
			Number of stocking events (*p*=0.511)			
			Number of fish stocked/ha (*p*=0.637)			
			Number of years since last stocking (*p*=0.134)			

Table S4.A11 **Pearson correlation between lake area and a proxy of Brook Charr density for a subsample of 28 lakes of the study. Density was estimated as the number of fish caught in per net per hour during sampling (catch per unit effort, for details, see Gossieaux et al. 2018).**

	Lake area
Brook Charr density	-0.22 [-0.55 – 0.17] p = 0.26

Degrees of freedom = 26.

Appendix B: References

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